UNIVERSIDADE CATÓLICA PORTUGUESA | INSTITUTO DE CIÊNCIAS DA SAÚDE

DENTAL IMPLANT SURFACE MODIFICATIONS AND OSTEOINTEGRATION

Dissertação apresentada à Universidade Católica Portuguesa para

obtenção do grau de Mestre em

Medicina Dentária

Por:

Valentina Andrea Ormazabal Toledo

Viseu 2013

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Acknowledgments

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Abstract

Osteointegration can be defined as the direct contact between the living bone and the implant surface without interposed soft tissue at the microscope level and it is a critical process to guarantee implant stability and consequent short and long term clinical success. Several factors are known to influence the efficiency of the osteointegration: bone status; loading conditions; implant design and material; implant surface and surgical technique.

Surface conditions are particularly important as they play a major role in the osteointegration process. Several characteristics among implant surface, such as surface composition, physic-chemical properties, surface wettability and roughness influence the rate and quality of osteointegration. Over the past three decades, the use of dental implants raised exponentially and widely expanded among the dental manufactures. The growing interest in improving the dental implant/bone interface has been addressed through the use of several techniques available for the modification of the surfaces dental implants in order to induce bioactivity. Different surface properties can induce different gene and protein expression in the osteogenic cells and also different structural and biomechanical properties to the surrounding mineralized tissue. This can result in different speed, quantity and quality of peri-implant bone formation. Nowadays, a growing aspect of endosseous implant surface research is focused on further enhancing the activity of bone forming cells at the tissue implant interface through the understanding of the fundamental processes at the interface between implant surfaces and surrounding living tissues.

The goal of this study is to review the contemporary knowledge about the influencing factors affecting the osteointegration process of dental implants, analyze the currently available techniques for implant surface modification and their limitations, and also discuss the future trends in surface bioengineering and nanotechnology for improving the osteointegration and consequently enhance their biological performance.

Key-words: Dental, Implant, Surface, Osteointegration, Osseointegration.

Resumo

A osteointegração, definida como sendo o contato direto estabelecido entre o tecido ósseo e a superfície de um implante com carga funcional, desconsiderando a interposição dos tecidos moles, é considerada como sendo uma fator crítico na manutenção da estabilidade do implante e, consequentemente, do seu sucesso clínico a curto e longo prazo. Vários são os fatores que influenciam a eficácia da osteointegração: o *status* do osso, as condições de carga, a anatomia e material do implante, as propriedades da sua superfície e a técnica cirúrgica utilizada. As propriedades da superfície do implante são um fator relevante no processo de osteointegração, na medida em que são várias as características das superfícies que afetam a quantidade, qualidade e a rapidez de concretização deste fenómeno. Sejam elas, a constituição da superfície, as propriedades físico-químicas, a molhabilidade e a rugosidade. A utilização de implantes dentários tem vindo a aumentar exponencialmente nas últimas três décadas, o que deu origem a uma rápida expansão do seu mercado. O crescente interesse em melhorar a qualidade da sua interface com o tecido ósseo, tem levado a desenvolver várias técnicas de modificação das superfícies implantares, procurando desta forma, induzir a sua bioatividade. Diferenças nas propriedades da superfície dos implantes revelam ter influência na expressão génica e na ação proteica ao nível das células osteoblásticas resultando, desta forma, em diferenças qualitativas e quantitativas na formação do tecido ósseo peri-implantar. Atualmente, os avanços na investigação de superfície de implantes endo-ósseos visam melhorar a atividade de células osteoblásticas na interface osso-implante. O objetivo do presente estudo consiste numa revisão do conhecimento atual sobre os fatores que influenciam o fenómeno da osteointegração, na análise das técnicas de modificação de superfície mais utilizadas, bem como das suas limitações. Pretende também discutir novas orientações em áreas como a nanotecnologia e bioengenharia de superfícies, avaliando de que forma podem estas áreas melhorar o seu desempenho clínico.

Palavras-chave: Dental, Implante, Superfície, Osteointegração, Osseointegração.

Table of contents

List of abbreviations

A

AA Arachidonic acid

AAT Anodized alkali treated

AL_2O_3 .Alumnina

Al-SL Alumina sandblasted

ALP Alkaline phosphatase

B

BCP-Ti Biphasic calcium phosphate

BIC Bone to implant contact

C

CaP Calcium phosphate

Coll Collagen

cpTi Commercially pure titanium

CS Chondroitin sulphate

E

EMD Emdogain -Enamel Matrix Protein

F

FH Fluoride

FN Fibronectin

H

HA Hydroxyapatite

HCl Hydrochloric acid

H2S04 Sulfuric acid

H3PO4 Phosphoric acid

hMSCs Human bone marrow mesenchymal stem cells

HNO3 Nitric acid

HF Hydrogen fluoride

I

IBAD Ion Beam Assisted Deposition

M

MC3T3-E1 Newborn mouse calvaria-derived cell line

MG63 Human osteosarcoma cells

MTS Mitochondrial activity

µm Micrometer

N

nm Nanometer

P

P-15 Biomimetic active peptide

PSHA Plasma Spray Hydroxyapatite

PGE 2 Prostaglandin E2

R

Ref References

rhBMP-4 Recombinant human bone morphogenetic protein-4

S

SEM Scanning electron microscopy

SLA Sandblasted Large Grit Acid etched

SBF Simulate body fluid

T

TiO2 Titanium oxide

Ti Titanium

TiZr Titanium- zirconium

TiNb Titanium- nobirium

TPS Titanium plasma spraying

TGF-b1 Transforming growth factor b1

X

XPS X-ray electron spectroscopy

Z

ZrO2 Zirconium dioxide

Dental implant surface modifications and osteointegration

Introduction

1. Introduction

The oral rehabilitation of missing teeth by dental implants is one of the most frequently used surgical procedures nowadays. The rate of clinical success of the use of oral implants is widely related to the bone formation at the endo-osseous implant surface in contrast with fibrous encapsulation that often leads to loss of the implant and consequent failure of the treatment. This process known as osteointegration was firstly described by Branemark in 1952 while examining microcirculation of bone and wound healing patterns, as the “direct contact between living bone and functionally loaded implant surface without interposed soft tissue, detectable at the microscope level” [1]. In 1990 he redefined the term as “ a continuing structural and functional coexistence, possibly in a symbolic manner, between differentiated, adequately remodeling, biologic tissues and strictly defined and controlled synthetic components providing lasting specific clinical functions without initiating rejection mechanism” [2]. It is a continuous process of formation and adaptation to function and repair, which takes place due to osteoblastic and osteoclastic bone activity, and is reflected in clinical features as an anchorage mechanism whereby non-vital components can be reliably incorporated into living bone and persist under physiological loading conditions [3]. Nowadays it is widely accepted by the scientific community that it is an absolute requirement for the successful implant-supported dental prosthesis. After decades of subsequent research in academia and industry, implants have evolved and now show high survival rates and good longevity. Research has resulted in better designs, materials, and more extensive clinical knowledge compared with the early years of implant development. However, the main cause for clinical failure is still insufficient bone formation around the biomaterial, immediately after implantation [4]. Therefore, improvements are needed in this area as clinicians and patients are pushing for faster healing times.

In 1981, Albrektsson and colleagues identified six parameter as pre-requisites for osteointegration: (1) bone status, (2) loading conditions; (3) surgical technique; (4) implant design (or macrostructure); (5) implant material and (6) implant surface.

1.1. Bone status

An absolute requirement for dental implant therapy is an acceptable supporting bone quality in terms of height, width, and density. The bone density available at the preliminary site for implant placement reflects a number of biomechanical properties, such as strength and modulus of elasticity and highly influences the treatment planning, implant design utilized, surgical approach and healing time required, and initial progressive loading during prosthetic reconstruction [5]. Also, the three requirements for primary stability of an implant, such as atraumatic bone preparation [6], close approximation of bone to the biocompatible implant surface [7], and absence of movement at the interface during healing [8] are closely related to bone density at the implant placement site. For this matter, an adequate bone diagnosis is mandatory for implant rehabilitation planning

Bone is an organ that is able to change in relation to a number of factors, including hormones, vitamins, and mechanical influences. However, biomechanical parameter such as duration of edentulous state are predominant [9]. Regardless of the high predictability of implant therapy, certain risk factors such as smoking, diabetes or periodontitis can predispose individuals to lower success rates due to their influence in wound healing [10]. Also, osteoporosis represents an important chronic disease in which bone density is affected by an excessively rapid degradation of hard tissue structure. According to the osteoporotic changes of the bone structure, a limited use of dental implants should be expected as there are unfavorable conditions for the primary stability, biological fixation and thus for the osteointegration of dental implant [11]. However, clinical review articles showed, that osteoporosis is not included as an absolute contraindication for implant surgery [12].

1.2. Loading

In order to fulfill the aesthetic and functional objectives over an extended period of time, a dental implant must be capable of withstanding the oclusal stresses generated in the oral environment and transfer this load to the supporting tissues in an appropriate direction and magnitude so tissue viability is maintained . In this respect, the implant principally acts to minimize and distribute the biomechanical forces which are characterized by their magnitude, duration, and type. The ability to transfer force largely depends on attaining interfacial fixation. The interface between the implant and bone must stabilize the biomaterial in as short a time as possible postoperatively, and once this condition is achieved, it must remain stable through a long lasting period [13]. Research led to the recognition of two types of implant stability that are mandatory to achieve osteointegration: primary and secondary [14]. Primary stability comes from mechanical engagement of the dental implant with cortical bone, and it is affected by the quantity and quality of the bone site where the implant is paced, surgical procedure, length, diameter, and shape of the implant. Secondary stability is developed from the regeneration and remodeling process of the bone and the implant surface [14].

1.3. Implant design

Several implant shapes have been developed in to improve the dental implant outcome and the importance of exact fit between bone and implant have been stressed by several authors [15]. Osteointegration is more easily achieved with cylindrical threaded or screw-shaped implants which are inserted so as to create maximal contact between bone and implant [16]. In an *in vitro* comparison between conical, natural tooth and cylindrical geometrical implant configuration the latter was shown to minimize the high stresses both in the implant and in the mandibular model tested [17]. The screw-shaped implants improves the achievement of primary stability minimizing undesirable early implant movements due to its threads that engage the bone in compression and transfer the applied load [18]. The thread

designs have been extensively researched to provide a minimum of shear forces and maximal compression to the bone which allows for the most favorable bone response. Furthermore, a screw-shaped implant provides an increased surface area for interaction between implant and tissue and is viewed, in this context, as a variant of the surface-porous implant system [19].

1.4. Surgical technique

Nowadays, there is a general consensus that before implant placement, prosthodontic planning should be performed as well as clinical and radiologic planning [20]. This means that the type and details of the final restorations should be considered prior to implant placement. A delicate surgical technique is essential to ensure osteointegration. All surgery requires gentle manipulation, tension-free closure, and obliteration of dead space [21]. It is necessary to maintain asepsis, cool the drill to avoid thermal necrosis, exert minimal pressure on bone and soft tissue, protect the blood supply, use of an adequate drill geometry and speed and careful tapping for the screws [22]. Adequate initial implant stability requires close evaluation of the bone quality as each osteotomy is prepared**.** As one of the most important goals is achieving a sufficient primary stability, the last drilling steps mainly depend on the type of bone [23].

1.5. Implant material

The biocompatibility profiles of synthetic biomaterials used for the replacement of biological tissues have always been a critical concern within the health care disciplines, especially in dental implant prosthetic reconstruction of the oral and maxillofacial areas due to the extension of the device from epithelial zones onto underlying bone. The physical, mechanical, chemical and electrical properties of the dental implant bulk material provide key inputs into the interrelated biomechanical and biological functions [24]. The implant should be manufactured from a tissue-tolerant material capable of withstanding the loads at the implantation site and having great corrosion resistance. A major issue for implant design is the development of materials that are physically and biologically compatible with alveolar bone. Ideally, bone should integrate with the material, substance, or device and remodel the bone

structure around it, rather than responding to the material as a foreign substance by encapsulating it with fibrous tissue.

In general, two basic types of materials are used in dental implants: ceramics and metals. The unique properties of ceramic materials, including their corrosion resistance and excellent esthetics, make them appealing candidates for many dental applications. However, their inferior mechanical properties, particularly their poor fracture resistance, have hampered their commercial use, especially in load-bearing situations [25]..

Titanium has commonly been used for the manufacture of dental implants over several decades due its properties such as excellent biocompatibility, low weight, high strength/weight ratio, excellent corrosion resistance, chemical stability low modulus of elasticity and easy shaping and finishing [26]. Dental implants are usually made from commercially pure titanium or titanium alloys and both exhibit superior mechanical properties, chemical stability, and in vivo biocompatibility than other biomaterials. Pure titanium is generally used when corrosion resistance is of higher importance than mechanical strength, whereas for instances the alloy Ti–6Al–4V, is used when mechanical strength and fatigue resistance is required [27]. An important property to take under consideration when using titanium dental implants in both titanium in his commercially pure form or as an alloy, is the oxide layer formed spontaneously in the surface, immediately after exposure to room temperature air and pressure. This passive layer is in straight contact with the body tissues playing an important role in corrosion resistance, biocompatibility and osteointegration [27, 28].

1.6. Implant surface

It is widely accepted that the surface properties of a dental implant play a major role in the osteointegration process and biomechanical fixation due to its influence in the implant-tissue interactions as it affects directly the behavior of the surrounding tissues [26, 29]. The surface features become extremely important at the initial healing period of an implant as they influence directly the

dynamics of the bone-implant interface and consequently command the short and long term success rate of the prosthetic treatment [30]. The implant surface characteristics including topography, chemistry, surface charge, and wettability are likely to be of particular relevance to the chemical and biological interface processes in the early healing stages after implantation. In fact, they play an extremely important role in the modulation of host/implant tissue response as it determines the speed and quantity of osteointegration and long-term survival of an implant [31] .Surface modifications influence cell proliferation and differentiation, extracellular matrix synthesis, local production factors, and even cell shape, gene expression, protein secretion, differentiation and apoptosis. This will consequently affect retention and proliferation of osteogenic cells at the implant site [32]. In addition, modified surfaces can also present osteoconductivity which allows for cell migration to the implant surface [33], promoting the formation of extracellular matrix and bone apposition.

During surgical preparation of the implant cavity, the integrity of the bone is interrupted, which leads, after placement of the dental implant, to a defined sequence of biological events, resembling fracture healing as they both begin with a breach in an intact skeletal site, an immune response, neo-vascularization, and recruitment of skeletal progenitor cells [2]. However in a fracture, some skeletal progenitor cells differentiate into chondrocytes, while others into osteoblasts, followed by endochondral ossification. Whereas, around an implant all skeletal progenitor cells differentiate into osteoblasts, followed by intramembranous ossification [34].Wound healing around a dental implant involves a highly orchestrated sequence of events which is triggered by tissue injury involving soluble mediators, blood cells, extracellular matrix and parenchymal cells .

After implant placement into a prepared osteotomy , three stages of repair occur : initial formation of a blood clot occurs through a biochemical activation followed by a cellular activation and finally a cellular response. This events can be subdivided into: hemorrhage into the defect with unspecific protein, adsorption by the dental surface, platelet activation and degranulation, inflammation, recruitment, migration, and adhesion of osteogenic progenitor cells (osteoconduction), osteogenic proliferation, osteogenic differentiation with

matrix synthesis, calcification (de novo bone formation), followed by lifelong bone remodeling at the implant surface [28]. Ultimately, it culminates in either partial or complete regeneration or repair. During surgery, dental implant surfaces interact with blood components from ruptured blood vessels and within a short period of time (Figure 1) , various plasma proteins such as fibrin get adsorbed on the material surface and the complement and kinin systems become activated [35]. The retention of these proteins by the implant surface is dependent upon the surface topography of the latter, and it is through this three-dimensional biological architecture that putative osteogenic cells migrate to the implant surface [35]. As in fracture healing, the migration of bone cells in peri-implant healing will occur through the fibrin of a blood clot. Since fibrin has the potential to adhere to almost all surfaces, it forms and serves as a scaffold for ingrowing capillaries, collagen fibers, mesenchymal stem cells and pre-osteoblasts at the implant surface [35]. However, as the migration of cells through fibrin will cause retraction of the fibrin scaffold, the ability of an implant surface to retain this fibrin scaffold during the phase of wound contraction is critical in determining whether the migrating cells will reach the implant surface. The activation of platelets occurs as a result of interaction of platelets with the implant surface as well as the fibrin scaffold and this leads to thrombus formation and blood clotting. Platelets, however, are of considerable importance since their activation leads to a rearrangement in cell shape and to centralization of storage granules followed by the release of their contents into the extracellular environment. This process of platelet degranulation releases a number of growth and differentiation factors which play a key role in the wound healing process by acting as signaling molecules for recruitment and differentiation of the undifferentiated mesechymal stem cells at the implant surface. Plasma also contains dissolved substances such as glucose, amino acids, various ions, cholesterols, and hormones which are needed for the viability of cells and tissues [34].

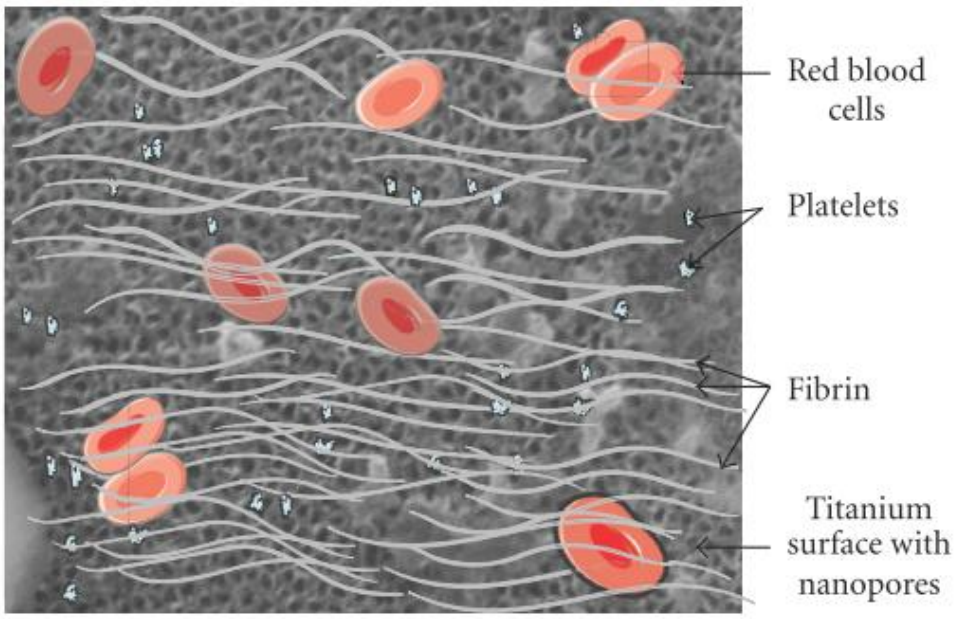


Figure 1 – Interaction of surface of dental implants with blood [35]

These cells initially remove the necrotic debris created by the drilling process and then undergo physiological changes which lead to expression of cell surface proteins and production of cytokines and pro-inflammatory mediators [36]. This cytokine-regulated cellular recruitment, migration, proliferation and formation of an extracellular matrix on the implant surface can be influenced by the macrophages. The end result of this complex cascade is promotion of a wound healing to finally start to form de novo bone on the implant surface.

The bone remodeling phenomenon occurs through the ability of osteoblastic cells to lie down on the old bone surface or on the implant surface itself and are described as distance and contact osteogenesis. In distance osteogenesis, new bone is formed on the surface of old bone in the peri-implant site that provides a population of osteogenic cells that lay down a new matrix that encroaches on the implant. In contact osteogenesis, new bone forms first on the implant surface as it becomes colonized by bone cells before bone matrix formation can begin (Figure 2) [33].

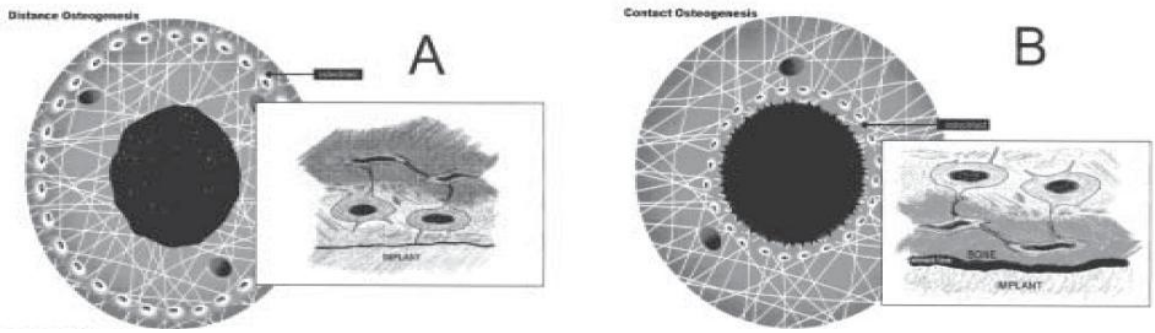


Figure 2 – Drawings to show the initiation of distance osteogenesis (A) and contact osteogenesis (B) where differentiating osteogenic cells line either the old bone or implant surface respectively. The insets show the consequences of these two distinctly different patterns of bone formation. In the former the secretorly active osteoblasts, anchored into their extracellular matrix by their cellular processes, become trapped between the bone they are forming and the surface of the implant. The only possible outcome is death of this cells. On the contrary, in contact osteogenesis, *de novo bone* is formed directly on the implant surface [33].

As surface characteristics modulates the outcome of cells behavior to the presence of a dental implant and subsequently the osteointegration level, the development of an implant surface that aims to attract osteoblasts that produce a bone extracellular matrix to ensure a high bone-implant contact has been the aim of several research studies over the last years.For this purpose, numerous surface engineering methods have been developed to create featured implant surfaces in order to improve the clinical performance of implants and to guarantee a stable mechanical bone implant interface [37]. Also, persistent efforts have been made in order to enhance the surface properties of dental implants to meet the increasing demands of implant treatments in an aging society and address the associated challenges, such as improving the success rate, expanding the applicability, and shortening the healing time required for sufficient bone-implant integration [38].

Implant surface topography refers to macroscopic and microscopic features of its surface, more specifically related to the degree of roughness and the orientation of the surface irregularities [39] and it can increase the surface contact between the bone and the implant, consequently improving the biomechanical interlocking between bone and implant [40, 41].

Besides, it enhances osteointegration due to the increased adhesion and differentiation of osteoblastic cells on this type of surfaces ,in contrast to fibroblasts and epithelial cells which adhere more strongly to smooth surfaces [42]. Numerous studies have shown marked differences in the *in vitro* and *in vivo* responses of textured implant surfaces demonstrating that the ability of the implants to support bone formation can be enhanced by modifying surface topography [28, 35, 36, 43].

In Figure 3, both machined (A) and micro-rough (B) dental implant surfaces [44] are presented.

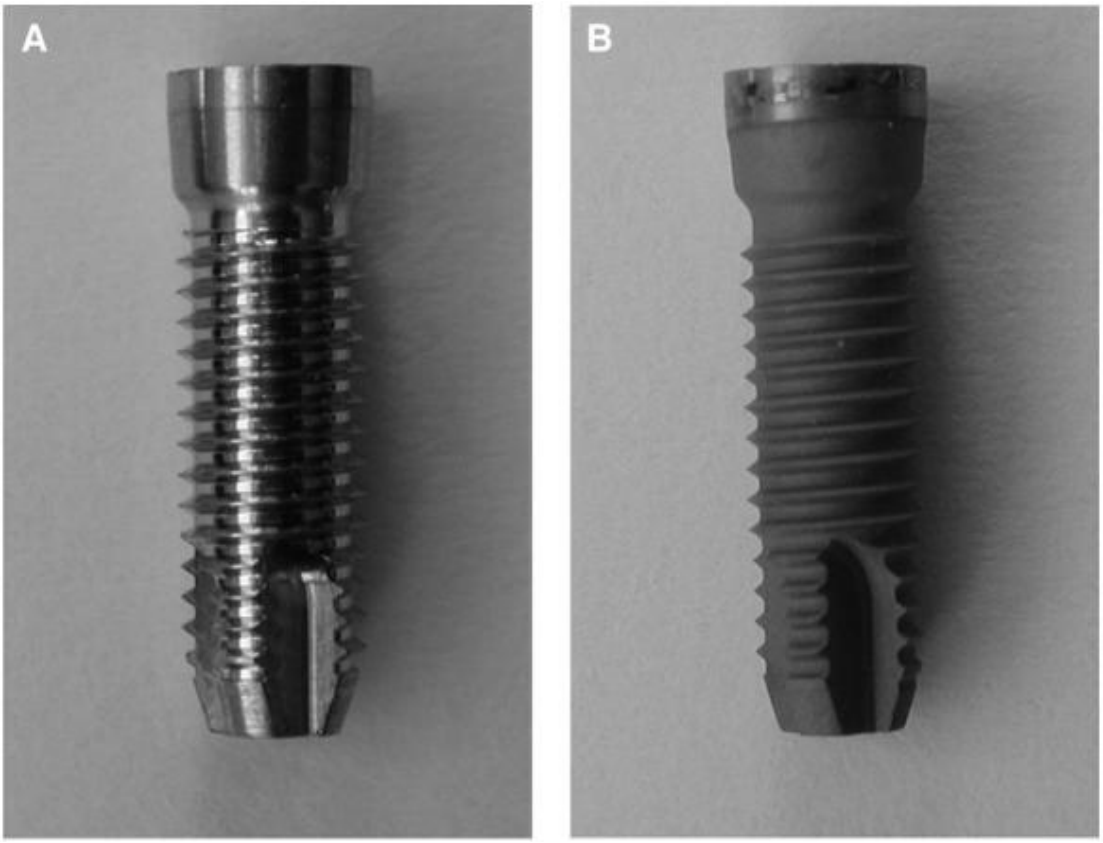


Figure 3 - Machined (A) and micro-rough (B) dental implant surfaces [44].

In figure 4, low magnification secondary emission micrographs of machined (A) and micro-rough (B) dental implant surfaces are presented.

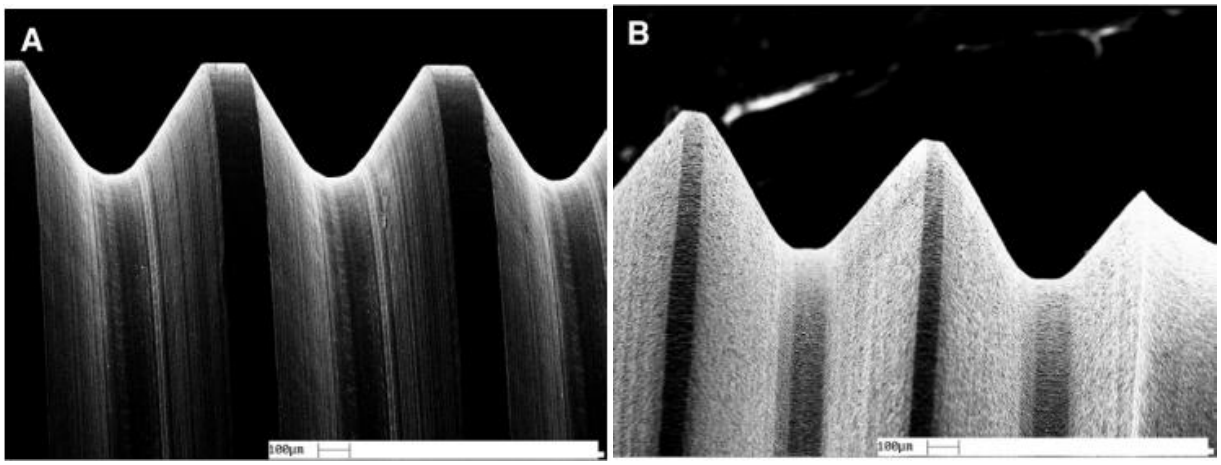


Figure 4 - Machined (A) and micro-rough (B) dental implant surfaces [44].

In vitro and *in vivo* studies have shown that titanium surface roughness influences a number of events in the behavior of cells in the osteoblastic lineage, including spreading and proliferation, differentiation, and protein synthesis [45]. Implant surface roughness is divided, depending on the dimension of the measured surface features into macro, micro, and nano-roughness, and each size of roughness provides contacts with different cells and biological molecules.

Macro-roughness comprises features in the range of millimeters to tens of microns. This scale directly relates to implant geometry, with threaded screw and macro porous surface treatments. Micro-roughness is defined as being in the range of 1–10 μm. Studies supported by clinical evidence suggest that this range of roughness maximizes the interlocking between mineralized bone and implant surface resulting in greater accrual of bone at the implant surface [46] . The majority of commercially available dental implants presents an average roughness of 1 to 2 μm [39].

In 2004, Albrektsson & Wennerberg proposed a definitions regarding dental implant surface topography that state smooth surfaces are comprised in the range of Sa < 0.5μm, minimally rough surface Sa 0.5- 1 μm (e.g., turned implants), moderately rough Sa 1–2 μm (e.g., acid etched, sandblasted or anodized) and rough Sa > 2 μm (e.g., plasma sprayed) [39, 47].

A nanostructure is an object of intermediate size between molecular and micrometre-sized structures. It involves materials that have a nano-sized topography or are composed of nano-sized materials with a size range between 1 and 100 nm. By definition, all surfaces show nano-topography, but not all of them have significant nanostructures [36]. At the nano-scale, a more textured surface topography increases the surface energy and consequently favors cell attachment and tissue healing, particularly directly after implantation. It might also directly influence cell proliferation and differentiation, because it has been suggested that nano-patterning can modulate cell behavior [36].

It has been shown that titanium implants with adequate roughness may influence the primary stability of implants, enhance bone-to-implant contact, and may increase removal torque force as they present a larger surface area and allow a firmer mechanical link to the surrounding tissues [48].

Surface chemistry influences the conformational changes in the structures and the interactive nature of adsorbed proteins and cells, which may lead to alterations in the structure of adsorbed proteins, generating a cascade of effects that may ultimately be evident at the clinical level and also affects the hydrophilic character of the surface. Surface wettability is largely dependent on surface energy and influences the degree of contact with the physiological environment [49] as they influence the adsorption of proteins, and increase adhesion of osteoblasts on the implant surface. The cell behavior on a hydrophilic surface promotes blood coagulation and higher expressions of bone-specific differentiation factors, in contrast with a hydrophobic surface. Consequently, dental implants' manufacturers have been developing highly hydrophilic and rough implant surfaces which in turn are able to exhibit better osteointegration than implants with smooth surfaces [50].

Nowadays, there are several methods to modify the implant surface characteristics with the main objective of improving the bio-mechanical properties of the implant such as stimulation of bone formation , removal of surface contaminants, and improvement of wear and corrosion resistance on rough surfaces from the macro- to the nanometric scale. Among these techniques, the most common are: Turned surface (machined dental implants), Gritblasting, Grit-blasting and acid-etching, Titanium Plasma Spraying, etcetera.

1.6.1. Turned or machined dental implant surface.

The first generation of dental implants, termed the turned implants, had a relatively smooth surface after being manufactured, are submitted to cleaning, decontamination and sterilization procedures [1]. This surfaces are usually and inadequately called “smooth” since scanning electron microscopy analysis showed that they have grooves, ridges and marks (Figure 5) derived from tools used for their manufacturing which provides mechanical resistance through bone interlocking [51]. However, the main disadvantage regarding the morphology of non-treated implants is the fact that osteoblastic cells are prone to grow along the grooves existing on the surface, which in terms of clinical implications means a longer healing time required [41]. The success rates of turned implants in challenging situations such as low bone density has been reported to be lesser than when placed in areas with good bone quality. Due to morphological characteristics and lower resistance to removal torque, machined dental implants are becoming commercially unavailable. Studies have shown lower primary stability for the turned implants, they demonstrated secondary stability values and clinical success rates similar to modified implants [30].

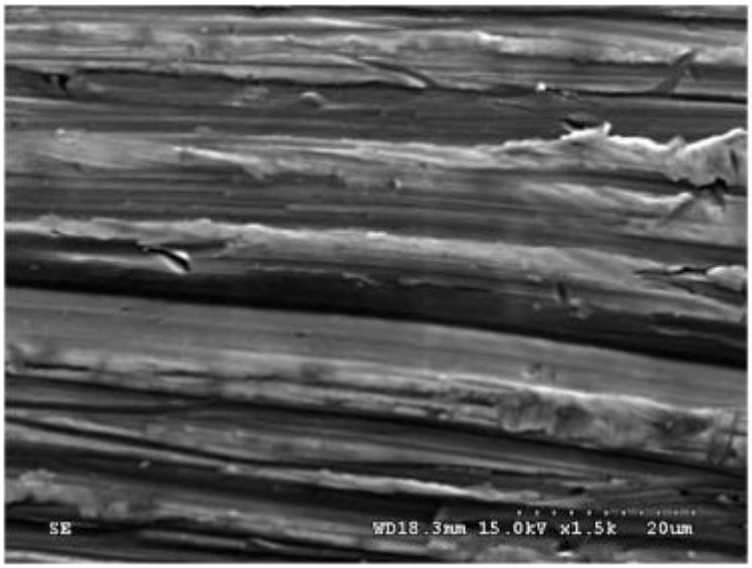


Figure 5 - Scanning electron micrograph of a machined implant surface [30].

1.6.2. Anodic oxidation.

In order to alter the topography and composition of the surface oxide layer of the implants ,micro- or nano-porous surfaces may also be produced by potentiostatic or galvanostatic anodization of titanium in strong acids, such as sulfuric acid, phosphoric acid, nitric acid and hydrogen fluoride at high current density or potential [52]. When strong acids are used in an electrolyte solution, the oxide layer will be dissolved along current convection lines and thickened in other regions which creates micro-or nano-pores on the titanium surface (Figure 6) [53]. This electrochemical process results in an increased thickness and modified crystalline structure of the titanium oxide layer. However, it is a complex procedure and depends on various parameters such as current density, concentration of acids, composition and electrolyte temperature [41].

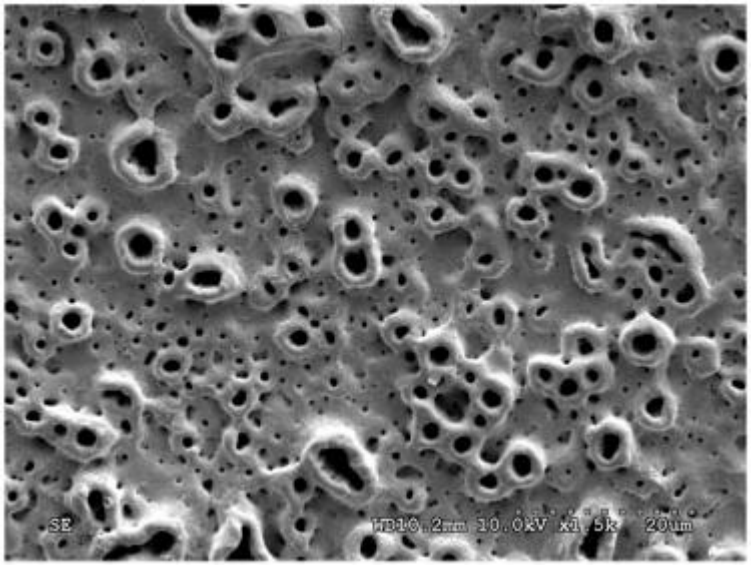


Figure 6 – Scanning electron micrograph of an anodized (TiUnite – Nobel Biocare) surface [30].

1.6.3. Grit-blasting.

Grit-blasting, consists in the propulsion towards the metallic substrate of hard ceramic particles that are projected through a nozzle at high velocity by means of compressed air and leading to different surface roughness, depending on the size of the ceramic particles (Figure 7) [29]. The grit blasting technique usually is performed with particles of silica (sand), alumina, titanium dioxide or resorbable bioceramics such as calcium phosphate [30]. Alumina (Al_2O_3) is frequently used as a blasting material, however, it is often embedded into the implant surface and residue remains even after ultrasonic cleaning, acid passivation and sterilization [29]. It has been documented that these particles have been released into the surrounding tissues and interfered with the osteointegration of the implants[54]. Moreover, this chemical heterogeneity of the implant surface may decrease the excellent corrosion resistance of titanium in a physiological environment [29]. Titanium oxide (TiO_2) particles with an average size of 25 µm can produce a moderately rough surfaces in the 1–2 µm range on dental implants [30].

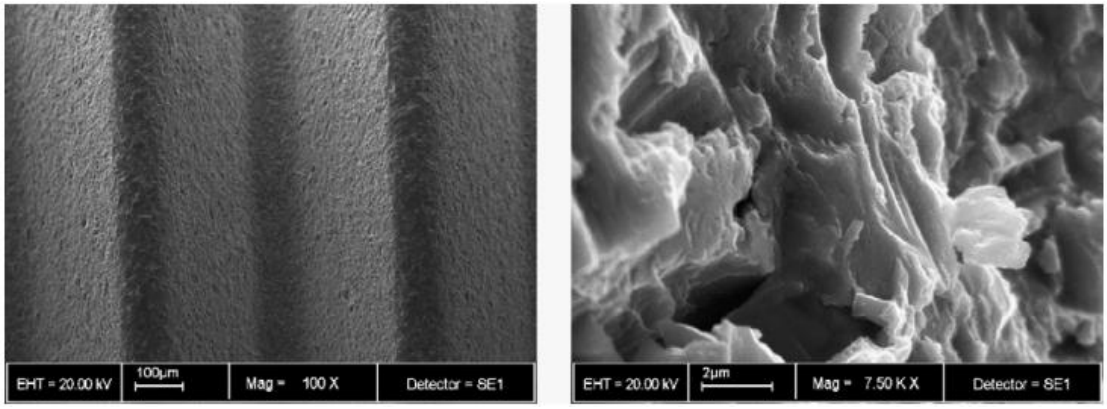


Figure 7 – Scanning electron micrographs of a TiO blasted surface (Astratech TiOblast, France) [29]

1.6.4. Acid-etching.

The immersion of a titanium dental implant in strong acids such as hydrochloric acid , sulfuric acid, nitric acid and hydrogen fluoride is another method of surface modification which produces micro pits on titanium surfaces with sizes ranging from 0.5 to 2 µm in diameter [55]. The resulting surface shows an homogenous roughness, increased active surface area and improved adhesion of osteoblastic lineage cells [56]. Dual acid-etching consist in the immersion of titanium implants for several minutes in a mixture of concentrated HCl and H2SO4 heated above 100 ◦ C to produce a micro-rough surface [29] (Figure 8) that may enhance the osteoconductive process through the attachment of fibrin and osteogenic cells, resulting in bone formation directly on the surface of the implant [57]. These studies hypothesized that implants treated by dual acid-etching have a specific topography able to attach to fibrin, improving the adhesion of osteogenic cells, and thus, promoting bone apposition [58]. On the other hand, acid-etching can lead to hydrogen embrittlement of the titanium, creating micro cracks on its surface that could reduce the fatigue resistance of the implants. Indeed, experimental studies have reported the absorption of hydrogen by titanium in a biological environment. This hydrogen embrittlement of titanium is also associated with the formation of a brittle hybrid phase, leading to a reduction in the ductility of the titanium wich is related to the occurrence of fracture in dental implants [29].

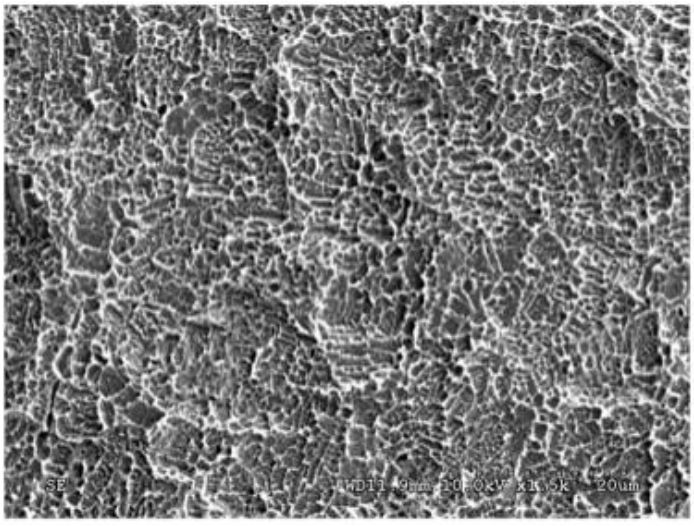


Figure 8 – Scanning electron micrograph of an implant surface processed through dual acid-etching procedure (Biomet-3i,Palm Beach Gardens, USA) [30].

1.6.5. Grit-blasting and acid etching.

Following grit-blasting , the surface is submitted to acid-etching to further enhance the topographic profile of the surface and remove processing byproducts [30]. The advantages of this method include an increase in the total surface area of the implant, achieved due to the selective removal resulting from electrochemical differences in the surface topography (Figure 9) [29]. Nevertheless, this process should be carried out under controlled conditions, as over-etching the surface decreases surface topography and mechanical properties and may be detrimental to osteointegration. In addition, it is important that the etching procedures following grit-blasting removes any particle remainaning, because chemical analyses of failed implants have shown evidence that the presence of such particles interferes with titanium osteoconductivity regardless of the established biocompatibility profiles of the biomaterial [59].

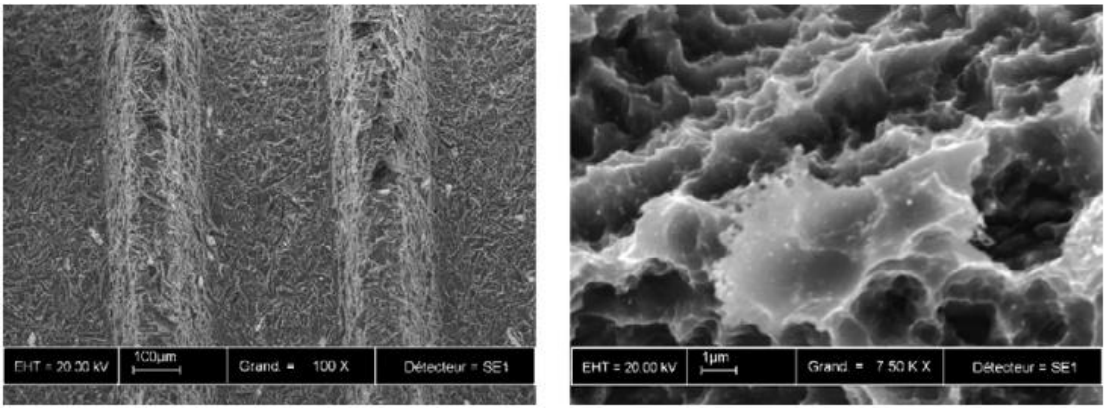


Figure 9 – Scanning electron micrographs of an SLA surface on Titanium dental implant (Straumann AG, Switzerland) [29].

1.6.6. Plasma-spraying.

Titanium plasma-spraying (TPS) consists in injecting titanium particles into a plasma torch at high temperature. This particles are projected on to the surface of the implants where they condense and fuse together, forming a film about 30 µm thick (Figure 10) resulting in an average roughness of around 7 µm [29]. The TPS processing may increase the surface area of dental implants up to approximately six times the initial surface area [60] and is dependent on implant geometry and processing variables, such as initial powder size, plasma temperature, and distance between the nozzle output and target [61]. One of the major concerns with plasma-sprayed coatings is the possible delamination of the coating from the surface of the titanium implant and failure at the implant-coating interface despite the fact that the coating is well-attached to the bone tissue. In a pre-clinical study using minipigs, the bone/implant interface formed faster with a TPS surface than with smooth surface implants presenting an average roughness of 0.2 µm. However, particles of titanium have sometimes been found in the bone adjacent to these implants [62]. However, while an increase of six times the original surface area may be a favorable scenario for bone growth and apposition it also becomes a risk factor when there is an exposure of the implant surface to the oral fluids and bacteria. In addition, a major risk with high surface roughness concerns difficulties in controlling peri-implantitis due to the intercommunication between porous regions facilitates migration of pathogens to inner bone areas, potentially compromising the success of the implant therapy [63].

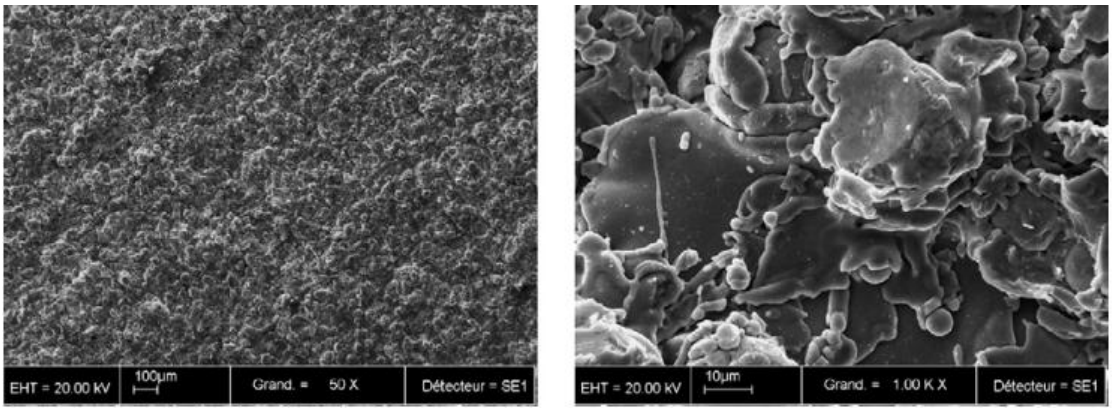


Figure 10 – Scanning electron micrographs of a Titanium plasma sprayed surface (Cam Implants BV, The Netherlands)[29].

1.6.7. Calcium phosphate coatings.

Calcium phosphate (CaP) coatings, mainly composed by hydroxyapatite, has been used as a biocompatible, osteoconductive and resorbable blasting materials [43] The idea behind the clinical use of hydroxyapatite is to use a compound with a similar chemical composition as the mineral phase of the bone in order to avoid connective tissue encapsulation and promote peri-implant bone apposition [64]. For this matter, the CaP coatings disclose osteoconductive properties allowing for the formation of bone on its surface by attachment, migration, differentiation and proliferation of bone-forming cells.

In the resorbable ones, following implantation, the release of calcium phosphate into the peri-implant region increases the saturation of body fluids and precipitates a biological apatite onto the surface of the implant [65].This layer of biological apatite might contain endogenous proteins and serve as a matrix for osteogenic cell attachment and growth [33] and therefore, improve osteointegration.

Plasma Sprayed Hydroxyapatite (PSHA) coatings are the most commonly found among the commercially available calcium phosphate coatings. The HA ceramic particles are heated to extremely high temperatures and deposited at a high velocity onto the metal surface where they condense and fuse together forming a 20–50 μm thick film (Figure 11) [64]. This resulting surface shows enhanced bioactivity observed at early implantation times, however, the mechanical resistance of the interface between the coating and titanium is considered to be a weak point, and some cases of implant failure have been reported [60]. Furthermore, it is recognized that regardless the resorbable blasting material, the release of particles of varied size from the surface may result in an inflammatory response detrimental to hard tissue integration [30].

Despite the substantially for PSHA-coated implants, this type of implant has fallen out of favor in dental practice as studies have shown that coatings do not uniformly dissolve/degrade after long periods in function.

Also, uniform coating composition and crystallinity have not always been achieved through the plasma spray process, and the overall literature database is controversial with respect to coating composition and crystalline content in relation to the *in vivo* performance [30].

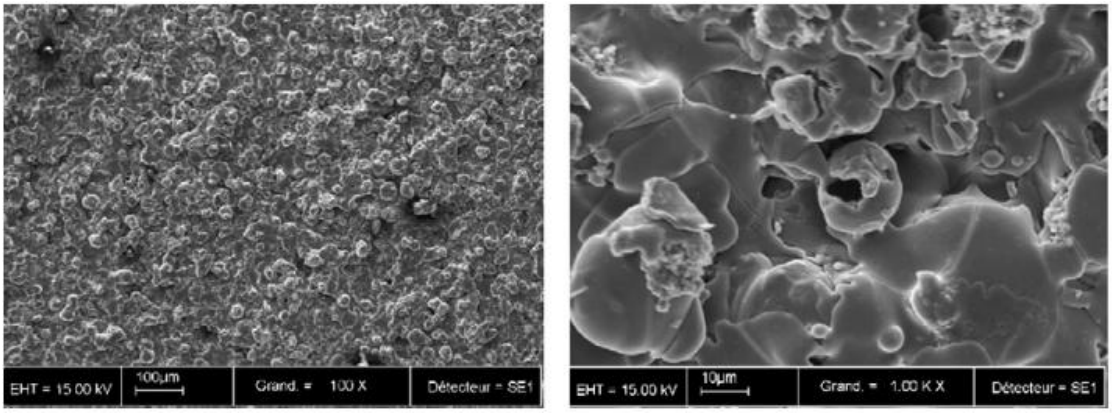


Figure 1 – Scanning electron micrographs of a plasma-sprayed hydroxyapatite coating surface (Cam Implants BV, The Netherlands) [29]

In order to improve PSHA coatings, a number of techniques have been developed with the aim of producing a thin-film nanostructured bioceramic coatings , such as sol-gel deposition, pulsed laser deposition, sputtering coating techniques, electrophoretic deposition and ion-beam-assisted deposition (IBAD) [30, 66]. These techniques may offer a more accurate compositional control and the possibility of fabricating much thinner layers (of the order of 1 µm or less). This could be advantageous for coating stability, as the driving force for cracking and delamination decreases with decreasing coating thickness [43]. Desirable features of thin-film coatings include coating controlled composition and thickness plus enhanced adhesion to the metallic substrate [67].

The Sol-gel electrophoresis method can be prepared using a dip coating or a spin coating process and is capable of improving chemical homogeneity in the resulting HA coating as it allows for better control of the chemical composition and macrostructure of the coating [41].

The Pulsed laser deposition results in a titanium surface microstructures with greatly increased hardness, corrosion resistance, and high degree of purity with standard roughness and thicker oxide layer [68].

The Ion-beam assisted deposition technology permits the formation of thin films at atomic and molecular levels, as well as low temperature syntheses utilizing ionic effects[69].

There is an increasing interest in the use of calcium phosphate in the dental implant surface coatings. However despite having a similar composition and chemistry to that of human bone, the mechanical properties of CaP´ s are far from being close to those of human bone, which limits their use for load-bearing applications. Recurrent drawbacks include controlling the calcium-phosphate layer composition, resorbability, weak adhesion to the substrates, the use of high temperatures or the costs involved in the process [70]. In fact, there are several reports of cracking and/or delamination of the coating due the generation of large thermal stresses during processing [43], which may affect the quality and rate of peri-implant bone formation [71].

1.6.8. Biomimetic calcium phosphate coatings.

Biomimetic coatings involves the use of microstructures and functional domains of organismal tissue function to deposit calcium phosphate upon medical devices in order to improve their biocompatibility [72]. This bioinspired method consist in the precipitation of calcium phosphate apatite crystals onto the dental implant surface through simulated body fluids under near-physiological or “biomimetic” conditions of temperatutre and pH.(Figure 12) [29].

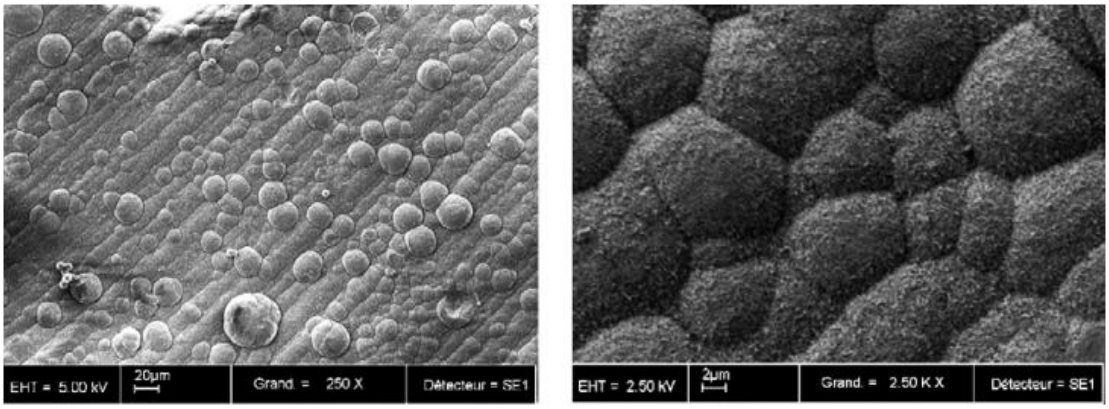


Figure 12 – Scanning electron micrographs of a biomimetic calcium phosphate coating [29].

Despite the considerable progress in dental implant research, there are still critical gaps in the knowledge about which and how surface properties determine the most adequate biological behavior of the surrounding tissue. Nevertheless it is clear that the tissue responses are mainly dictated by processes controlled at the nano-scale level. However, current surface chemistries and morphologies are mostly controlled, at the micron level.

Understanding and controlling interfacial reactions at the nano-level is still an open scientific challenge, and the answer could be extremely useful to rationally design implant surfaces and consequently obtain more predictable clinical results.

This work aims at providing a systematic review of the state-of-the-art related to dental implant surfaces and the recent advances on improving the quality of bone its interface through strategies of osteointegration and regeneration. A comparative analysis will be performed reporting several examples of surface modification strategies both at the pre-clinical and clinical stage.

Materials and method

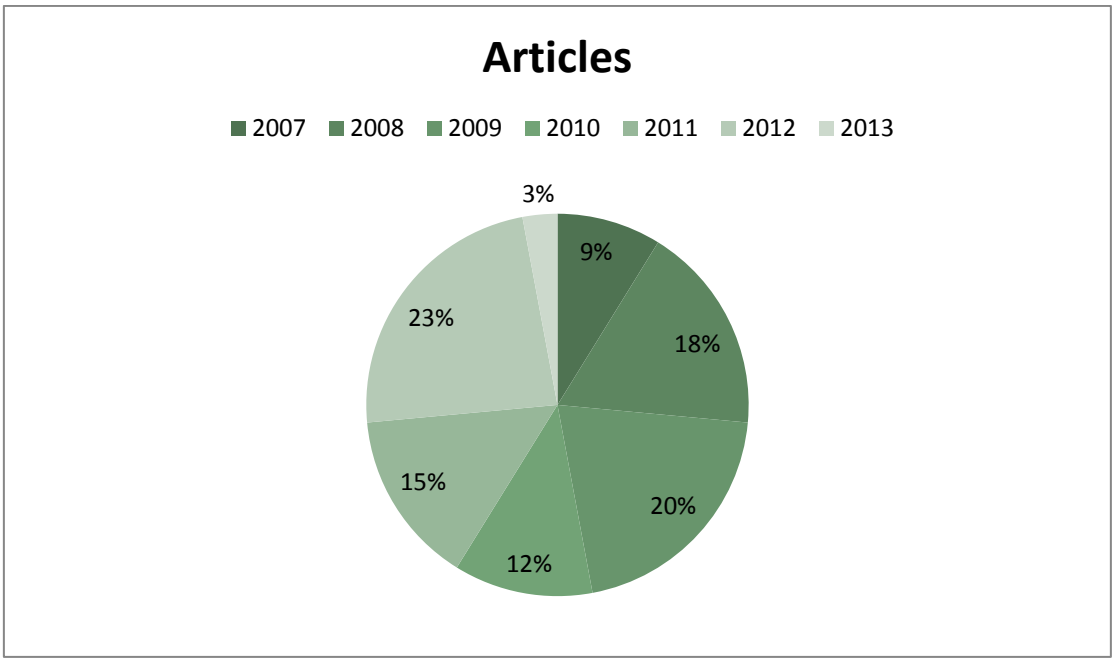
2. Materials and method.

An exhaustive search was made to select and extract data from PubMed and ScienceDirect electronic databases for the period comprised between December 12 of 2012 and February 14 of 2013. In a first stage, the keyword combination “Implant AND dental AND surface AND osteointegration NOT review” was selected for the search. Some authors use a different terminology for “osteointegration”: “osseointegration”. To make sure that all the relevant papers were included, the same keyword combination was used including this term: “Implant AND dental AND surface AND osseointegration NOT review”. Papers covering the period from January 2007 and February 2013 were selected for further revision. The non-English written manuscripts were excluded. The searches yielded 74 articles from Pubmed, and 70 from ScienceDirect: during the combinations of results from both searches, 68 duplications removed. The remaining 76 articles were examined. The inclusion criteria were as following: Physicochemical studies of novel dental implant surfaces , *In vitro* studies including cell activity in different dental implant surfaces, *in vivo* performance of various implant surfaces, clinical trials related to osteointegration and clinical behavior from different methods of implant surface modifications, studies including immobilization of bioactive molecules to improve osteointegration, biomimetic coatings of implant surfaces using nanoparticles or growth factors. The exclusion criteria were: case reports and case studies, book chapters, conference proceedings, *in-vivo* studies with a small amount of samples (less than 5) and *in vitro* studies including other agents that do not use bone-related cells, physicochemical evaluation of currently commercially available surfaces.

Results

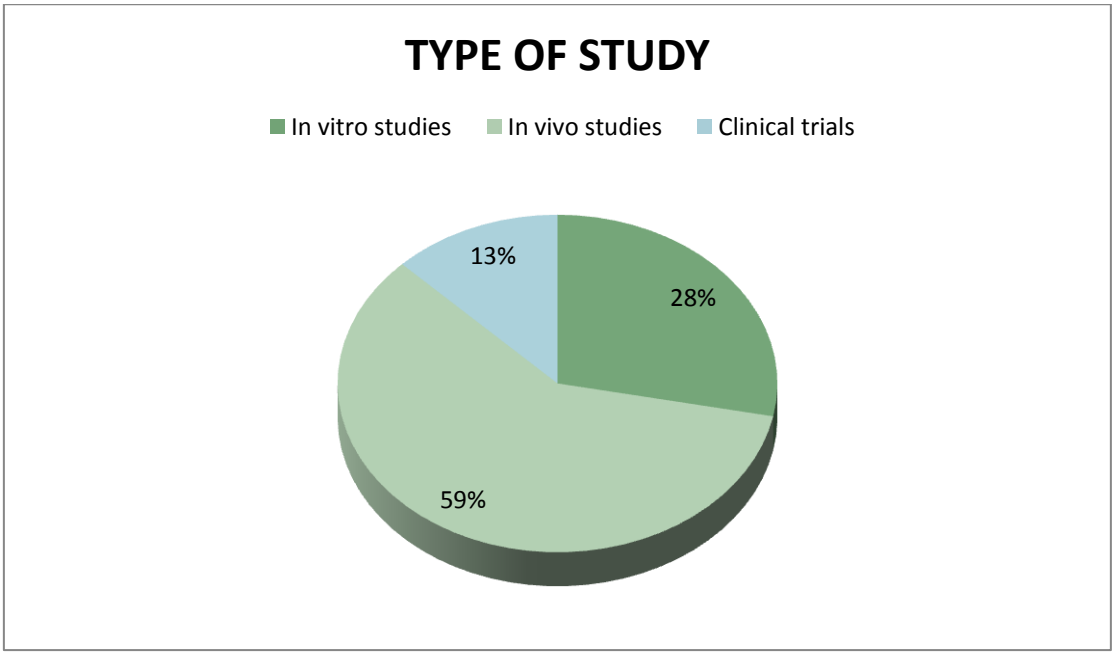
3. Results

76 studies were identified and from these, 32 related studies met the inclusion criteria and were considered for the present systematic review. Graphic 1 represents the percent of relevant studies published per year between January 2007 and March 2013. It is noted that most of the papers concerning the aim of this study were published in 2012 and 2009.



Graphic 1 - Percentage of relevant papers published *per* year between January 2007 and February 2013.

In graphic 2 is represented the type of studies that matched the inclusion criteria that are in a initial physicochemical phase, *in vitro* , *in vivo* and clinical trial. From the 32 papers analyzed, 59 % represented *in vivo* studies, 28 %, *in vitro* studies , and 13 % represented Clinical studies. The majority of papers found were *in vivo* studies, followed by *in vitro* studies and in a very small amount, clinical trials



Graphic 2 – Percentage of type of study that includes up to physicochemical studies, *in vitro* studies, *in vivo* studies and clinical trials published between January 2007 and February 2013.

3.1. Clinical trials.

The clinical studies were included in Table 1 [73-76]. The commercially available implants included anodized surface modifications were tested using as a control group, implants with machined surfaces [75, 76]. SLA implant surface [74] , titanium surfaces coated with hydroxyapatite and bioactive glass [74] behavior were also analyzed. In Table 1, Clinical trials are presented with their respective bulk material, patients characteristics, type of surface tested, roughness, methodology and outcome.

Table 1 – Clinical trials

Ref.	Bulk	Patient characteristics	Surface	Sa	Methodology	Outcome
Mystry *et al.*[73]	Titanium alloy (Ti-6Al-4V)	Thirty-one systemically healthy, partially edentulous patients (19 males and 12 females, age range 18– 56 years, average age 36 years)	Ti coated with HA Ti coated with Bioactive Glass	Not specified Not specified	Bioactive glass and HA was coated on Ti alloy. Hydroxyapatite coating was applied on the implant surface by air microplasma spray technique and bioactive glass coating was applied by vitreous enamelling technique. The outcome was assessed up to 12 months after prosthetic loading using different clinical and radiological parameters.	Hydroxyapatite and bioactive glass coating materials were non-toxic and biocompatible. The overall results showed that bioactive glass coated implants were as equally successful as hydroxyapatite ones in achieving osteointegration and supporting final restorations
Kim *et al.* [74]	Commercially pure (cp) Titanium (Grade 4)	Sixty-five systemically heathy patients (36 males and 29 females)	SLA	Moderately rough	From June 2003 to February 2005, 65 patients were enrolled and cumulative survival was calculated based on Kaplan-Meier method. to confirm prognosis factor and Cox proportional hazard model was established.	5-year cumulative survival rate of tapered implants with SLA surface was 95.41%
Van Assche *et al.*[75]	Commercially pure (cp) Titanium (Grade 4)	Eighteen patients (eight smokers and five women with mean age at implant insertion: 55.4 years old) in 2005–2006	Turned implant Anodized (TiUnite – Nobel Biocare)	Minimally rough Moderately rough	Two subgroups of patients were formed; one group (n = 10) where all teeth had been extracted due to severe periodontitis, another group (n = 8) with teeth in the antagonistic jaw with a history of periodontitis. 4 to 7 turned and anodized implants were inserted randomly in each patient. After 3–6 months peri-implant parameters and intra-oral radiographs were recorded up to 1 year after abutment connection.	Two turned implants failed in the partial edentulous group during the initial healing period and none of the TiUnite surface. No statistically significant differences in clinical parameters could be observed between both surfaces.
Quirynen *et al.*[76]	Commercially pure (cp) Titanium (Grade 4)	Eighteen patients (eight smokers and five women with mean age at implant insertion: 55.4 years old) in 2005–2006	Turned implant Anodized (TiUnite)	Minimally rough Moderately rough	Two subgroups of patients were formed; one group (n = 10) where all teeth had been extracted due to severe periodontitis, another group (n = 8) with teeth in the antagonistic jaw with a history of periodontitis. 4 to 7 turned and anodized implants were inserted randomly in each patient. Subgingival biofilm formation was followed up for 1 year, and samples were analyzed by culture technique, qPCR and checkerboard.	Over the entire period, no statistically significant differences could be detected in subgingival microbiota between the minimlly and moderately rough surfaces showing that the roughness of the more modern implants did not influence the biofilm formation during the first year of implant loading. In partially edentulous patients, the biofilm matured to a higher concentration of pathogens when compared with fully edentulous patients.

3.2. Pré-clinical studies

3.2.1. *In vivo* studies

In vivo studies were included in Table 2. The sandblasted and acid etched surface modification technique was the most tested *in vivo* studies [31, 77-83], followed by anodized surface modifications [31, 44, 78, 79, 84-86], calcium phosphate coatings [77, 78, 81, 84, 87, 88], acid etching, grit blasting [79, 80, 82, 89, 90], type I Collagen coating [91], electropolished surface [88], titanium plasma spray [89], zirconium sandblasted [89], laser treated [92]. Machined surfaces were mainly used as control group [31, 44, 78-80, 82, 86, 89, 90, 92, 93] as Non trated surfaces [94]. In Table 2, the *in vivo* studies that matched the inclusion criteria are presented with their respective bulk material, animal model and implantation time utilized, type of surface tested, roughness, methodology of study and outcome.

Table 2 – *In vivo* studies

Ref.	Bulk	Animal model implantation time	Surface	Sa	Method	Outcome
Albouy et al. [79]	Titanium Grade not specified	Dogs, 1 year old. 5 months .	Turned surface (Biomet 3i) SLA (Straumann) Grit Blasted (TiOBlast – Astra tech) Anodized (TiUnite – Nobel Biocare)	Minimally rough Moderately rough Moderately rough Moderately rough	Four implants representing four different implant systems were placed in the mandible of six dogs. Three months after implantation t, experimental peri-implantitis was initiated by placement of ligatures and plaque formation. The ligatures were removed when about 40–50% of the supporting bone was lost. Four weeks later, surgical therapy including mechanical cleaning of implant surfaces was performed. No systemic antibiotics or local chemical antimicrobial therapy were used. After 5 months, block biopsies were obtained and prepared for histological analysis.	Two of the TiUnite (anodized) implants were lost after surgical therapy. Radiographic bone gain occurred at implants with turned, TiOblast (grit-blasted) and SLA surfaces, while at TiUnite implants additional bone loss was found after treatment. Resolution of peri-implantitis was achieved in tissues surrounding implants with turned and TiOblast surfaces.
Albouy *et al.* [86]	Titanium Grade not specified	Dogs,1 year old. 6 months	Turned surface Anodized (TiUnite– Nobel Biocare)	Minimally rough Moderately rough	Four implants with similar geometry and with two different surface characteristics (turned surface and anodized surface - TiUnite; Nobel Biocare AB) were placed pair wise in one side of the mandible in five dogs, 3 months after tooth extraction. Experimental peri-implantitis was initiated by placement of ligatures and plaque formation. The ligatures were removed when about 40% of the supporting bone was lost. After 6 months, block biopsies were obtained and prepared for histological analysis.	The amount of bone loss that occurred during the plaque accumulation period after ligature removal was significantly larger at implants with a TiUnite surface than at implants with a turned surface. The histological analysis revealed that the vertical dimensions of the lesion and the pocket epithelium and the apical extension of the biofilm were significantly larger at TiUnite implants than at turned implants.
Elias *et al.* [31]	Commercially pure (cp) Titanium (Grade 4)	Rabbit, average weight ± 6 kg. 12 weeks	Turned surface Sandblasted and acid-etched Acid-etched Anodized	Minimally rough Moderately rough Moderately rough Moderately rough	Eight dental implants different surfaces were inserted in the tibia of the rabbits. The implants were removed 12 weeks after the surgery, and the peak removal torque was recorded using a digital torquimeter.	The results show that the surface roughness and wettability of implants may influence biological responses such as the removal torque of dental implants. The highest contact angle and the lowest removal torque were presented by machined implants. The lowest contact angle and the highest removal torque were presented by the anodized dental implants.
Kim *et al.* [83]	Commercially pure (cp) Titanium (Grade 4)	Rabbits (male), average weight 3.0–3.5 kg, 9 months old. 4 weeks	Sandblasted and acid-etched	Minimally rough	The animal tests were carried out using screw-shaped Ti implants sandblasted and then acid etched. After a healing period of four weeks, the rabbits were sacrificed and bone blocks were obtained to perform histological analysis and calculate the contact ratio between bone and implant.	The histological images show new bone formation over the implant surface. Moreover, the bone grew down along the implant surface from the cortical bone to the apex.
El Wassefy *et al.* [85]	Commercially pure (cp) Titanium (grade 2)	Rabbit, 10 to 12 months, average weight 3.0-3.5 kg 2 and 4 weeks.	Anodized	Moderately rough	The biomechanical evaluation was performed to verify the effect of the anodized surface modification on the interface resistance to shear force. Routine histological analysis was performed to evaluate the bone tissue reactions to the studied surface.	The implant–bone interface resistance to shear force was enhanced at 2-week healing period.
Chiesa *et al.* [44]	Commercially pure (cp) Titanium (Grade 2)	Sheep, 3-5 years old with average weight of 7 ± 5 kg. 4,8 and 12 weeks	Turned surface Acid- Etched Anodized and alkaline treated	Minimally rough Moderately rough Moderately rough	Twelve sheep were subjected to bilateral fixture implantation in the femoral condyles (one fixture for each surface treatment for each femoral condyle). Four animals were pharmacologically killed at 4, 8, and 12 weeks after surgery, and the femurs were excised into cubic bone segments, each one containing an implant, and blind histomorphometric evaluation was performed.	Both *in vitro* and *in vivo* results highlighted that both, acid etched and AAT sufaces had a better performance compared with turned Ti implants. The AAT treatment improved the osteointegration at 4 weeks after surgery and achieved the greatest osteointegration at 8 weeks, which implies the possibility of an improvement in adhesion and proliferation of osteoblastic cells.

Table 2 – *In vivo* studies continued

Ref.	Bulk	Animal model implantation time	Surface	Sa	Method	Outcome
Gil *et al.* [82]	Commercially pure (cp) Titanium (grade 3)	Minipigs. 3 days, 1, 2, 3 and 10 weeks	Turned surface Acid-etched Grit-blasted Grit-blasted and alkaline-etched	Minimally rough Moderately rough Moderately rough Moderately rough	Three hundred and twenty implants with four different surface modifications were placed into the bone of 20 mini-pigs. The percent of bone-to-implant contact was determined 3 days, 1, 2, 3 and 10 weeks after implant placement by histomorphometric analysis. Surface composition, topography and wettability of the implant specimens were analyzed.	The combination of shot-blasting and thermo-chemical treatment accelerated bone regeneration at early stages in comparison with all other treatments between day 3 and week 3. The value of osteointegration attained at week 2 was maintained until the end of the experiment without any significant changes. This was mostly attributed to the ability of these implants to form in vivo a layer of apatitic mineral that coated the implant and could rapidly stimulate bone nucleation and growth from the implant surface.
Aparicio *et al.*[80]	Commercially pure (cp) Titanium (Grade 3)	Minipigs, 6 years old. 2,4,6 and 10 weeks	Turned surface Grit-Blasted Acid-etched Grit-blasted and alkaline-etched	Minimally rough Moderately rough Moderately rough Moderately rough	The percent of bone-to-implant contact after 2, 4, 6, and 10 weeks of implantation as well as the mechanical retention after 4, and 6 weeks of implantation were evaluated with histometric and pull-out tests, respectively, as a measure of the osteointegration of the implants.	The grit-blasted and alkaline-etched surface treatment produced micro-rough and bioactive implants that accelerated bone tissue regeneration and increased mechanical retention in the bone bed at short periods of implantation in comparison with all other implants tested
Koh *et al.* [78]	Commercially pure (cp) Titanium (Grade 4)	Rabbit, average weight 2.5-3.5 kg 2 and 4 weeks.	Turned surface SLA Anodization Anodization + CaP immersion	Minimally rough Moderately rough Moderately rough Moderately rough	Three test groups were prepared: SLA implants, anodized implants, and anodized implants with CaP immersion. The turned implants served as control. Twenty rabbits received 80 implants in the tibia. Resonance frequencies were measured at the time of implant insertion, 2 weeks and 4 weeks of healing. Removal torque values were measured 2 and 4 weeks after insertion	The surface-modified implants appear to provide superior implant stability to the turned one. However, neither anodic oxidation nor CaP immersion techniques showed any advantage over the conventional SLA technique with respect to implant stability
Fontana *et al.*[84]	Titanium Grade not specified	Rabbit, adults, average weight around 4.0 – 5.0 kg 2, 4 and 12 weeks.	Porous oxide surface Anodization + CaP immersion	Not specified Moderately rough	Each rabbit received six implants. Animals were sacrificed after 2, 4 and 9 weeks of healing.. The femoral implant and the proximal implant of the tibia of each animal were subjected to the histologic analysis and the distal implants of the tibia underwent removal torque test	Ca–P coating had no beneficial effect in improving bonding strength at the bone–implant interface either at 2, 4 and 9 weeks.
Jimbo *et al.* [87]	Commercially pure (cp) Titanium (Grade 4)	Rabbits , average weight 4.5 kg. 6 weeks	Turned surface CaP coated : Smooth surface Without discernable crystal structures of phosphorous deficient HA CaP coated: needle-shaped HA crystal layer Cap coated: calcium deficient HA crystals	Minimally rough Nanofeatured Nanofeatured Nanofeatured	Stable CaP nanoparticle suspensions of different particle sizes and structures were coated onto implants by immersion and subsequent heat treatment. An uncoated implant was used as the control. After topographical and chemical characterizations, implants were randomly inserted into rabbit tibiae for removal torque testing.	The CaP coatings with nanostructures on the implant surfaces had enhancing effects on osteointegration. Along with the surface nanotopography, the CaP chemistry might have influenced the biological outcome..
Svanborg *et al.*[81]	Commercially pure (cp) Titanium (Grade 3)	Rabbits, adults . 2,4 and 9 weeks	Sandblasted and Acid etched Sandblasted and acid-etched + Single coat of HA Sandblasted and acid-etched + Double coat of HA	Moderately rough Moderately rough Moderately rough	Sandblasted and acid etched titanium implants coated with two different thicknesses of hydroxyapatite (test implants) and sandblasted and acid etched titanium implants (control implants), were inserted in rabbit tibia. After a healing time of 2, 4 and 9 weeks, a removal torque analysis and a histological evaluation were performed.	The results from the removal torque analysis showed a tendency for higher values of BIC for the double coated hydroxyapatite after 4 weeks and for both the coated surfaces after 9 weeks of healing. The histological evaluations indicated an slightly increased bone formation with the coated implants compared with the control. However the differences did not reach statistical significance.

Table 2 – *In vivo* studies continued.

Ref.	Bulk	Animal model implantation time	Surface	Sa	Method	Outcome
Lutz *et al.*[77]	Commercially pure (cp) Titanium (grade not specified)	Pigs, 18 months old. 14 and 30 days	Grit blasting and acid-etched +electrochemical deposition HA Grit blasting and acid-etched +electrochemical deposition HA +biomimetic peptide (P-15) concentration of 20 µg/ml Grit blasting and acid etched +electrochemical deposition HA +biomimetic peptide (P-15) concentration of 200 µg/ml	Not specified Not specified Not specified	The surfaces of dental implants were grit-blasted, acid-etched and then coated with HA. Experimental implants were further coated with a biomimetic active peptide (P-15) using two different concentrations. These biofunctionalized samples and control implants with no peptide were placed in the forehead region of 12 adult pigs. Six animals were evaluated for a period of 14 or 30 days.	Histomorphometric analysis demonstrated that the implants with the higher concentration of P-15 had significantly higher percentage of bone-to-implant contact at 14 and 30 days compared with the other groups. Both concentrations of P-15 showed increased peri-implant bone density compared to the control group at 30 days.
Meireles *et al.* [88]	Commercially pure (cp) Titanium (Grade 3)	Rabbit, 10 months old. 4 weeks .	Nano-hydroxyapatite electropolished surface Electropolished surface	Nanofeatured Smooth	One of each implant was placed in the rabbit tibia in a surgical site 0.7 mm wider than the implant diameter, resulting in a gap of 0.35 mm on each implant side and implant stability was ensured by a fixating plate. Topographical evaluation performed with an optical interferometer	The results from this study were not able to support nanometer HA as a bioactive coating to enhance bone formation in a gap design.. Chemically modified implants with nano-HA resulted in similar bone growth compared to control implants with similar nanotopography in early stage evaluation.
Bjursten *et al.* [90]	Commercially pure (cp) Titanium (grade not specified)	Rabbit, adults, 1 year old, average weight 3.0-3.5 kg 4 weeks	Titanium dioxide nanotubes Grit blasted	Nanofeatured Moderately rough	The implants were placed in eight rabbits and after 4 weeks, pull-out test and histological analysis were performed in order to indicate bone bonding strength to the implant surface and bone-implant contact.	TiO2 nanotubes significantly improved bone bonding strength compared with TiO2 gritblasted surfaces. Histological analysis confirmed greater bone-implant contact area, new bone formation, and calcium and phosphorus levels on the nanofeatured surfaces.
Rong *et al*. [92]	Commercially pure (cp) Titanium (grade not specified)	Rabbit, adults, average weight 2.5-3.0 kg 4 weeks	Turned surface Acid-etched Laser-treated Laser-treated and acid-etched.	Minimally rough Moderately rough Not specified Not specified	A total of 56 screw-shaped implants were grouped as follows: group A: implants were turned surface; group B: implants were laser-treated surface; group C: implants were acid-etched; group D: Implants were laser-treated and acid-etched surface. After 4 weeks, the removal torques and Bone-implant-contact were evaluated.	The removal torque and bone-to-implant contact measurements yielded statistically significant differences between the modified surface groups and turned group. The laser-treated and acid-etched surface achieved higher BIC than the laser-treated surface. There was no statistically significant difference between the laser-treated and acid-etched surface and the acid-etched surface in bone-to-implant contact.
Bacchelli *et al*. [89]	Commercially pure (cp) Titanium (Grade 2)	Sheeps 3.0 ± 0.5 year old, average weight 70 ± 5.0 kg. 2, 4 and 12 weeks	Turned surface Zirconia sandblasted Titanium plasma sprayed Alumina sandblasted	Minimally rough Rough Rough Rough	Twelve sheep were divided into three groups of four animals each and underwent implant insertion in tibia cortical bone under general anesthesia. The implants with surrounding tissues were subjected to histology, histomorphometry, scanning electron microscopy and micro-hardness tests	The experimentation indicated that at 2 weeks Zr-SL implants had the highest bone ingrowth compared to the other implant surfaces, and newly formed bone inside the threads was significantly higher than machined surface. The ZrO 2 treatment showed better results in peri-implant newly formed bone than machined and TPS processing, whereas its performance is similar to the Al-SL surface treatment
Stadlinger *et al*. [91]	Commercially pure (cp) Titanium (Grade 3)	Mini-pigs, 1 year old with averafe weight of ± 7 kg. 6 months	Type I collagen Coll/CS Coll/CS/rhBMP-4	Not specified Not specified Not specified	Each miniature pig had six implants (two of each coating) endosseously inserted in a randomized trial. Six months after implantation the animals were sacrificed and the implants removed in an bloc section for histomorphometric investigation of BIC.	The present results in fact show that rhBMP-4 adsorbed to a coll/CS coating not only does not enhance BIC but even shows a statistically significantly lower BIC compared to the same coating without pre-integrated rhBMP-4.
Nikolidakis *et al.* [95]	Commercially pure (cp) Titanium (Grade 3)	Goats, average weight ± 60 kg. 6 weeks	Acid-etched Acid-etched loaded with 0.5 µg TGF-b1 Acid-etched loaded with 1.5 µg TGF-b1	Moderately roug Not specified Not specified	Twenty-four cylindrical screw type implants were used and TGF-b1 in two different concentrations was applied on sixteen of them. Each animal received three implants: one Ti (control), one Ti loaded with 0.5 µg TGF-b1 (Ti-TGF0.5), and one Ti loaded with 1.0 µg TGF-b1 (Ti-TGF1.0). The eight animals were euthanized at 6 weeks after implantation and implants with surrounding tissue were retrieved for histological preparation and histomorphometrical evaluation	Light microscopical analysis showed the occurrence of an intervening fibrous tissue layer around about half of the TGF-b1 loaded implants. Further, the histomorphometrical measurements revealed that the Ti implants demonstrated the highest percentage of bone-implant contact while Ti-TGF 1.0 implants showed the lowest amount. The difference between these two groups was statistically significant.

3.2.2. *In vitro* studies.

The *in vitro* studies on implant surface are included in Table 2. The anodization [85, 96, 97] and SLA [54, 98-100] surface modification techniques were the most commonly found among the in vitro studies, followed by acid etching[98, 101] , biphasic calcium phosphate [54], grit blasted [98], zirconium/niobium coating [102] ,hydroxyapatite coating [93] and fluoride coatings [101].The non-treated/turned surfaces [54, 96, 98-100, 102]were mostly used as a control group.

In table 3, *in vitro* studies are presented including their respective bulk material, type of surface studied, roughness, methodology utilized and outcome.

Table 3 – *In vitro* studies

Ref.	Bulk	Cell type	Surface	Sa	Method	Outcome
Yang et al. [97]	Commercially pure (cp) Titanium (grade not specified)	Human bone marrow mesenchymal stem cells (hMSCs)	Anodization	Nanofeatured	A fast electrochemical anodization treatment, applying different anodic currents, was used to produce a nano/submicron-scale network oxide layer on Ti metal surface. The anodized Ti surface was analyzed using thin film X-ray diffractometer, X-ray photoelectron spectrometer, and field emission scanning electron microscope. The blood coagulation and human bone marrow stem cells (hBMSCs) adhesion on the anodized Ti surface were evaluated.	This TiO2 network layer significantly enhanced the blood coagulation and human bone marrow stem cell adhesion.
Chiang *et al.* [96]	Commercially pure (cp) Titanium (grade not specified)	Human bone marrow mesenchymal stem cells (hMSCs)	Smooth Ti Anodization	Smooth Nanofeatured	Surface characterization of the network layer was carried out using thin film X-ray diffractometer and field emission scanning electron microscopy. Human bone marrow mesenchymal stem cells (hMSCs) were made to express green fluorescent protein (GFP) by retroviral transduction	The TiO 2 nano-network layer on the anodized Ti surfaces significantly improved *in vitro* and *in vivo* hMSC growth relative to hMSC growth on untreated Ti surface.
Le Guehennec *et al.*[54]	Commercially pure (cp) Titanium (Grade 3)	Newborn mouse calvaria-derived cell line MC3T3-E1	Smooth Ti Alumina blasted and acid etched (Alumina Ti) SLA Biphasic calcium phosphate, grit-blasted and acid-etched	Smooth Rough Moderately rough Rough	Cell viability of osteoblastic-cells cultured for 4, 8 and 15 days on the different titanium surfaces was measured by mitochondrial activity (MTS) and compared.	The results show that osteoblastic cells attached, spread and proliferated more rapidly on smooth surfaces than on rough surfaces while their differentiation was enhanced by rough morphologies. Concerning the rough surfaces, all tested surfaces were cytocompatible regardless of the blasting material used. MTS activity increased more rapidly on SLA and BCP–Ti than on Alumina–Ti and Smooth-Ti. Similar osteoblastic cell behavior was observed on BCP-blasted and SLA surfaces.
Park *et.al.* [93]	Commercially pure (cp) Titanium (grade not specified)	Human osteoblast cells	Turned surface HA coating using aerosol deposition without post-heat treatment. HA coating using aerosol deposition with post-heat treatment.	Minimally rough Minimally rough Minimally rough	Cell proliferation or attachment on the HA-coated Ti surface was assessed using scanning electron microscopy (SEM).	HA coating using aerosol deposition without post-heat treatment has a good biocompatibility, and provides a promoting strategy to enhance osteointegration in the application of dental implants
Elias *et al*. [101]	Commercially pure (cp) Titanium (Grade 4)	Human osteoblast cells	Acid-etched. Acid etched + fluoride ion modification. (NanoPorous) Acid etched + human plasma fibronectin incorporation (Porous) -Acid etched + fluoride ion modification+ human plasma fibronectin Incorporation	Moderately rough Nanofeatured Moderately rough Nanofeatured	Two titanium dental implants (acid etching and nano-acid etching followed by fluoride ion modification) were characterized by high-resolution scanning electron microscopy, atomic force microscopy, and X-ray diffraction before and after the incorporation of human plasma fibronectin (FN). The biofunctionalization of these surfaces and their effects on the interaction with osteoblastic cells where examined	The evaluation techniques used showed that the Porous and Porous-Nano implants have similar microstructural characteristics. Spectrophotometry demonstrated similar levels of fibronectin adsorption on both surfaces (80%). The association indexes of osteoblastic cells in FN-treated samples were significantly higher than those in samples without FN. The radioactivity values associated with the same samples suggested that FN incorporation is an important determinant of the in vitro cytocompatibility of the surfaces.

Table 3 – *In vitro* studies continued.

Ref.	Bulk	Cell type	Surface	Sa	Method	Outcome
Dean *et al.*[98]	Commercially pure (cp) Titanium (Grade 2)	MG63 human osteosarcoma cells (because they show several characteristics of Immature osteoblasts	Smooth Ti Smooth and acid etched Rough Ti Rough acid etched	Smooth Moderately rough Rough Rough	Four different types of commercially pure titanium (cpTi) disks with surfaces of varying roughness were prepared. MG63 osteoblasts were seeded onto the surfaces, cultured to confluence, and then treated for the last 24 hours of culture with AA and PGE-2	Both AA and PGE 2 influence osteoblast response by promoting osteoblast differentiation on smooth surfaces, while inhibiting it on rough surfaces.
Miron *et al.*[99]	Commercially pure (cp) Titanium (Grade 2)	Rat calvarial osteoblasts	Smooth Smooth+EMD SLA SLA + EMD	Smooth Smooth Moderately rough Moderately rough	Smooth and SLA titanium discs were coated with Emdogain (EMD) or left uncoated. Primary rat calvarial osteoblasts were cultured on each surface from 1 h to 4 weeks.	EMD enhances osteoblast differentiation on Ti surfaces, in a topography-independent manner
Yang *et al.* [100]	Commercially pure (cp) Titanium (Grade 3)	Mouse pre-osteoblast cells (MC3T3-E1)	Turned surface Sandblasted and acid-etched + immersion in sinvastatin solution.	Minimally rough Moderately rough	The control group consisted of cells cultured on titanium disks without any intervention for different time intervals (4, 7, and 14 days), and the experimental groups (simvastatin-loaded groups) consisted of cells cultured on titanium disks that were preincubated in varying concentration of simvastatin for the same time intervals of the control group. Alkaline phosphatase (ALP) activity, type I collagen synthesis, and osteocalcin release were used to measure the cellular osteoblastic activities	All simvastatin-loaded groups showed increased ALP activity compared with the control group at every time point. In the type I collagen synthesis assay all simvastatin-loaded groups showed an increase, and the effect was inverse dose dependent. This stimulatory effect of simvastatin was also observed in the osteocalcin release assay.
Sista *et al.* [102]	Commercially pure (cp) Titanium (Grade not specified)	Mouse pre-osteoblast cells (MC3T3-E1)	Titanium (Ti) Ti-Zirconium (TiZr) Ti-Niobium (TiNb	Smooth Smooth Smooth	The morphology,chemical analysis, surface roughness, and contact angle measurements of the alloys were assessed by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), profilometer, and contact angle goniometer, respectively whereas the biological properties of the materials were evaluated by measuring the adhesion, proliferation, and differentiation of MC3T3-E1 osteoblast cells on the surfaces of these alloys	The results showed that TiZr has a better biological profile than Ti or TiNb based on the initial attachment of MC3T3-E1 osteoblast cells on these materials.

Discussion

4. Discussion.

The available dental implant surface modifications techniques presented in Tables 1,2 and 3 are widely diverse but with a common goal which is to achieve better osteointegration and as a consequence, improve the implant lifetime. This review summarizes the recent advances on dental implant surface modification that are already at the clinical stages and compare with those still at preclinical stage of development, namely *in vivo* and *in vitro* studies. Some limitations are inevitable in this review. The search has been restricted to the use of PubMed and Science Direct engines. On the other hand, the used terms for the search were quite specific. These might decrease the number of papers that met the inclusion criteria. Nevertheless the obtained results constitute a representative collection of the most relevant techniques for the surface modification of dental implants.

In spite of many creative and interesting findings and methodologies for dental implant surface engineering, there are still plenty of limitations and questions to be overcome and answered.

4.1. Clinical trials.

Mistry *et al*. [73] compared the short-term performance of hydroxyapatite coated and Bioactive glass coated Ti-6Al-4V dental implant in each subject. The overall results showed that Bioactive glass coated implants are as equally successful as hydroxyapatite coated implants in achieving osteointegration and to support final restorations under the present experimental conditions. Equally important was the fact that it did not cause extra biological complications and therefore is safe to be used in humans. However, as the sample size of the present study is reduced, and the period of time for testing was very limited, further similar studies are required to throw more light on the observations made in this study and to come to a more solid conclusion.

Kim *et al*. [74] analyzed the risk factors for 5-year loaded tapered implants with SLA, showing a cumulative survival rate of 95.41%. These results showed that this commercially available surface has a positive outcome.

However, it would be interesting to compare the long term survival rate with other surfaces also available on the market.

Van Assche *et al*. [75] compared anodized (TiUnite) and turned implant surfaces in patients with a history of periodontal disease. Two turned implants failed in the partial edentulous group during the initial healing period whereas none of the anodized surface failed. However, no statistically significant differences in clinical parameters could be observed between both surfaces showing that moderately rough implants have a similar clinical outcome to smooth surfaces. These results are contradictory to the vast documented data presented in the past and present literature that state that the micro-rough implants have better outcome than smooth machined dental implants.

Quirynen *et al.* [76] compared the subgingival microbiota around turned and anodized (TiUnite) implants and abutment surfaces in both partially and fully edentulous patients susceptible to periodontitis. This study failed to demonstrate a significant relationship between surface roughness and microbial adhesion, both qualitatively and quantitatively. As peri-implantitis seems to be a condition that might need some time before it develops, long-term data are necessary before the above-mentioned can be fully accepted and extrapolated to the long-term. Significant differences were observed between partially and fully edentulous patients, with the former being at increased risk, independently from a minimally or moderately rough implant surface

Both previous studies [75, 76] evaluate the clinical behavior of anodized implants and turned implants within periodontally compromised patients and the results indicate that the behavior of anodized implant surfaces did not show statistically significant differences comparing with the turned surface.

Clinical evaluation comprises the most challenging testing protocol due the need to evaluate a large number of subjects in order to represent significant results. Furthermore, despite the reasonable amount of different surfaces currently available in the market, there is very limited data concerning the long-term outcome of each one of them, and almost any data that compares them in equivalent clinical situations. The differences between the surgical approach, characteristics of control groups, times of study, loading protocol and host status make the comparison between different studies rather difficult. Moreover, a proper analysis to the interface is not possible since the implants will not be retrieved from the patient. Therefore, for comparative purposes animal studies represent an excellent opportunity to gain as much knowledge about the impact of a certain surface modification technology on promoting a proper osteointegration.

4.2. Pré-clincial studies

4.2.1. *In vivo* studies.

Albouy *et al*. [79] analyzed the effect of surgical treatment of peri-implantitis without the use of systemic antibiotics at different types of dental implant surfaces commercially available , such as machined (Biomet 3i), Grit blastes - TiOblast (Astra Tech AB), SLA (Straumann AG) and anodized - TiUnite (Nobel Biocare AB) surfaces. Radiographic bone gain occurred at implants with turned, grit blasted (TiOblast) and SLA surfaces, while at anodized (TiUnite) implants, additional bone loss was found after surgical treatment. Resolution of peri-implantitis was achieved in tissues surrounding implants with turned and grit blasted (TiOblast) surfaces whereas remaining inflammatory lesions were found in SLA sites. No signs of resolution were found in sections representing anodized (TiUnite) implants. The results of this study reveals that the resolution of peri- implantitis following treatment without systemic or local antimicrobial therapy is possible and the outcome of therapy is influenced by implant surface characteristics.

A year later, the author analyzed the spontaneous progression of induced peri-implantitis in turned surface and anodized (TiUnite – Nobel Biocare) surfaces [86]. Radiographic bone loss was more pronounced at implants with a TiUnite surface than at implants with a turned surface and the vertical dimensions of the inflammatory lesion at the pocket epithelium and the apical extension of the biofilm were also significantly larger at the anodized implants.

These findings that indicate increased spontaneous progression of peri-implantitis in implants with a specific surface modification points to the need of analyzing risks with such modifications in relation to peri- implant diseases.

In periodontally compromised cases, the anodized surface showed in both of these studies [79, 86] and in clinical trials [75, 76] to have no influence in the healing process. These papers points to the importance of risk assessments in treatment planning and the need to further investigate the problem related to decontamination of implant surfaces.

Elias *et al*.[31] studied the relationship between surface properties such as roughness, wettability and morphology of commercially available titanium dental implant and removal torque. The highest contact angle and the lowest removal torque were presented by turned implants, whereas the lowest contact angle and the highest removal torque were presented by the anodized dental implants. The removal torques of dental implants with treated surfaces showed to be higher than those with turned surfaces, due the greater union between the *de novo bone* and the micro-roughness surface on the implant, which in overall outcome means that the osteointegration mechanism are also higher in this surfaces.

As it has been widely documented [41, 48, 103], machined surfaces have consistently showed a lower bioactivity and overall performance than modified surfaces. Due to the surface morphology characteristics and the smallest resistance to removal torque, the implants without surface treatment, denoted as machined or turned implants, are presently in disuse, as they are being removed from the market.

Kim *et al.* [83] tested the biocompatibility of SLA-treated titanium implants and concluded that the SLA surface showed good biocompatibility with both *in vitro* and *in vivo* studies showing an excellent survival rate (98.7%) with an average marginal bone loss of 0.28 mm over a period of 15.2 months. This results are as promising as showed in a clinical trial performed by the same author [74]. However, it would be interesting to compare the clinical and pre-clinical behavior of this surface with other commercially available ones.

El-Wassefy *et al.* [85] Tested the behavior of a bioactive dental implant surface modification achieved by anodization in sulfuric acid solution followed by thermal treatment. They concluded that anodization of titanium implants produced morphological changes, raised the percentage of oxygen in the TiO_2 layer, increased surface area and roughness of implants remarkably, and modified the crystallinity of the film. And, when surgically implanted into animals, showed higher initial resistance to shear forces and better

osteointegration, which may be advised in immediate loading protocols. However, in order to know the real potential of this new bioactive surface, there is a need to assess the surface for longer periods of time and to compare its behavior with other commercially available dental implant surface modifications. Since there are reports of compromised behavior of anodized surface in cases of periodontal disease [75, 76, 79, 86], it would be interesting to evaluate if this bioactive modification of the current surface has any influence in this matter.

Chiesa *et al.* [44] developed a new dental implant surface modification to enhance the osteointegration, designated AAT (for anodized alkali-treated titanium) and compared with a simply machined surface and an acid- etched surface. He suggested that the three tested implant surfaces could be suitable for endosseus implants. However the machined surface and the acid etched surface,both clean and decontaminated, were found to support cell adhesion and viability without enhancing proliferation whereas the AAT surface proved to have a potential influence in this last parameter.

Gil *et al.* [82] assessed the short-term bone regenerative potential of new osteoconductive surface for implants which combines grit-blasting with a thermo-chemical treatment. Theis modification showed to significantly accelerate the osteointegration compared with grit-blasted, acid-etched and machined implants.

Aparicio *et al.* [80] assessed the short- and mid- term bone regenerative potential and mechanical retention of a novel Two-Step surface modification consisting in sandblast and alkaline-etched+thermally-treated surface and compare it with grit-blasted, acid-etched and machined titanium implants. The new Two-Step surface produced micro-rough and bioactive implants that accelerated bone tissue regeneration and increased mechanical retention in the bone bed at short periods of implantation in comparison with all other implants tested.

The three previously referred studies [44, 80, 82] include the use of alkaline modifications on different surfaces. The overall results were quite promising as they showed substantial improvements in achieving fast and

stable osteointegration of endosseus implants which could be a major tool when immediate loading is required. However, in order to further evaluate these surfaces, long-lasting studies are mandatory.

Koh *et al.* [78] evaluated the biomechanical characteristics of various implant surfaces, such as SLA, anodized, anodized with posterior calcium phosphate immersions and control machined surface. They concluded that surface modifications of SLA, CaP and anodized surface showed faster osteointegration and bone healing than machined surface. Under the limitation of this study, they suggested that neither anodic oxidation nor CaP immersion techniques had any advantage over the conventional SLA technique with respect to implant stability.

Fontana *et al*. [84] researched the effects on the osteointegration of endosseous implants of a calcium phosphate coating on an anodized surface compared with a porous oxide surface. The results showed that by means of BIC and removal torque values, suggested that the CaP coating had no effect in improving bone apposition and interfacial strength at the bone–implant interface.

Jimbo *et al*. [87] evaluated the biological effects of three calcium phosphate coatings with similar nanostructures on relatively smooth implant surfaces. The results of the current study showed that the biological outcomes was influenced by the addition of CaP coatings and the three different CaP coatings with nanostructures on the implant surfaces had enhancing effects on osteointegration. Along with the surface nanotopography, the CaP chemistry might have influenced the biological outcome. As described in the introduction section, it its widley accepted that the chemical composition of the nano-coatings is one of the decisive factors for osteointegration [35, 36, 38, 64].

Svanborg *et al.* [81] investigated if nanometer thick coatings of hydroxyapatite nano-crystals applied on a moderately rough surface might enhance early bone healing on screw-shaped dental implants. This study did not support the importance of nanometer thick coatings of HA nanocrystals in early bone healing, when applied on a blasted and etched surface and placed in

a cortical bone. Further studies are needed to evaluate if and how nanostructures could be of importance in the bone healing process.

Lutz *et al*. [77] investigated the biofunctionalization of titanium implants with a biomimetic active peptide (P-15). The effect of P-15 concentration compared with controls was evaluated to determine the optimal amount of P-15 to insure optimal osteointegration. The surface modifications included Grit blasting and acid etching and electrochemical deposition HA; Grit blasting and acid etching and electrochemical deposition HA coated with biomimetic peptide (P15) concentration of 20 µg/ml ; and Grit blasting and acid etching and electrochemical deposition HA coated with biomimetic peptide (P15) concentration of 200 µg/ml. To date, biomimetic coatings have only led to minor improvements of implant healing and osteointegration [41, 64]. This may result from unspecific binding of cells to the implant surface leading to suboptimal results. The peptide P-15 sequence provides a highly specific binding site for osteoblast integrins , and therefore has a positive effect on bone regeneration when combined with bone substitute materials or carrier materials like hydroxyapatite. This investigation showed a significant positive effect of the biomimetic peptide P-15 at a concentration of 200 mg/ml on the BIC. The low concentration of the biomimetic peptide seemed to be insufficient to increase the BIC, as the BIC rates in group in this group were comparable with that of the control group. Additional studies are ongoing to determine the optimal concentration of the biomimetic active peptide and to demonstrate faster bone formation compared with implants without the P-15.

Meireles *et al.* 2008 [88] investigated the possible bioactivity of nano-hydroxyapatite bioactivity in a gap design study by comparing early bone healing to nanoscale hydroxyapatite-coated electropolished cylinders and electropolished cylinders without coating. The two implant surfaces investigated in this study had similar nanotopography but different surface chemistry, and the early in vivo response revealed similar bone contact and bone area values in a gap model. The results from this study were not able to support nanometer hydroxyapatite as a bioactive coating to enhance bone formation in a gap design with very smooth implant. Chemically modified implants with nano-

hydroxyapatite resulted in similar bone growth compared to control implants with similar nanotopography in early stage evaluation.

The previous studies [77, 78, 81, 84, 87, 88] evaluated the potential of biomimetic calcium phosphate coatings on different implant surfaces with contradictory results. Different sizes, shapes and distribution of nanostructures should be evaluated and efforts toward finding a standardized method for characterization of these structures should be developed to compare studies more easily. It is clear that additional studies are also important to investigate if nanostructures could influence bone healing in conditions of poorer bone quality and if they could have a significant effect on the clinical treatment of patients.

Bjursten *et al.* [90] investigated the *in vivo* bone responses to titanium dioxide nanotubes surface compared with grit blasted surface and indicated that TiO_2 nanotubes significantly improved bone bonding strength compared with TiO_2 gritblasted surfaces. Histological analysis confirmed greater bone-implant contact area, new bone formation, and calcium and phosphorus levels on the nanotube surfaces. It is anticipated that further studies will contribute to a better understanding of the effect of implant nano-topography on in vivo bone formation and bonding strength.

Rong *et al.* [92] evaluated the early osteointegration of laser-treated and acid-etched surface, laser-treated , acid-etched and machined surface. They observed a significant enhancement of new bone apposition to the laser-treated and acid-etched surface during the early stages of bone regeneration and concluded that this approach showed better osteointegration than the laser-treated surface. However, further researches in laser-treated and acid-etched surface are still needed.

Baccelli *et al***.** [89] evaluated the efficacy of zirconium dioxide sandblasting in comparison with other surface modifications currently used, such as machined surface, titanium plasma spray and alumina sandblasted (Al-SL) at short- and medium-term experimental times. Although Al2O3 is the most widely used material in the family of engineering ceramics,ZrO2 is suitable by virtue of its hardness and resistance to wear.

Zr-SL implants show a better osteogenesis than turned surfaces at all the experimental times analyzed, and also with respect to the TPS implants at 12 weeks. This paper indicate that the surfaces of the Zr-SL implants have a similar performance to the Al-SL devices in terms of bone ingrowths. However, their bone-to-implant contact was inferior and the micro-hardness results confirmed that the bone adaptation around Zr-SL implants was similar to that of Al-SL implants. Further *in vivo* investigations using an experimental model mimicking clinical applications will shed more light on the mechanical binding between bone and ZrO2 treated. The delamination process should also be assessed, as in Al-SL it is described as one of the major concerns in long-term implant survival [54].

Stadlinger *et al.* [91] Observed *in vivo* whether collagen coatings enriched by chondroitin sulphate and rhBMP-4 had a positive influence on bone-to-implant contact compared to collagen coatings alone. In order to create surfaces that are beneficial to the osteointegration, components of the natural cell surroundings were employed in this study. Type I collagen that is a major component of the extracellular matrix, known for its low immunogenicity and its binding properties for osteoblastic cells and has been shown to increase the rate of bone formation compared to an uncoated titanium implant and favor the adhesion and differentiation of osteoblastic cells [104]. Collagen-coated implants may be modified by the inclusion of the glycosaminoglycan chondroitin sulphate (CS) that has the ability to act as an intelligent scaffold, and interacts with endogenous growth factors that affects bone formation by the induction of osteogenic cell proliferation and growth factor binding. Coll/CS-coated implants may also be enriched by the growth factor rhBMP-4 to induce bone formation.

The results of histomorphometric measurement shows the highest level of bone formation on the overall implant surface for coll/CS implant coatings. The osteointegration of purely collagen-coated implants showed to be lower but not statistically different. The rhBMP-4 adsorbed to a coll/CS coating not only did not enhance BIC but even showed a statistically significant lower BIC compared to the same coating without pre-integrated rhBMP-4. This last result was mainly related to the concentration of growth factor applied.

The positive effect of coll/ CS indicates that the addition of other ECM components to collagen coatings could provide further benefits for the osteointegration of implants, although this will have to be further tested. The use of small amounts of growth factor requires an extensive research in order to become a viable tool to improve osteointegration in the future.

Nikolidakis *et al.* 2008 [95] investigated the effect of a low dose of transforming growth factor b1 (TGF-b1) incorporated through direct absorption to the surface implant and its effect during the early bone-healing period. Within the limitations of this study, it can be concluded that a burst released low dose of TGF-b1 has a negative influence on the integration of oral implants in trabecular bone during the early post-implantation healing phase. Therefore, the application of a low dose of TGF-b1 on an oral implant surface, which is subsequently delivered by burst release, cannot be recommended for clinical application.

A high diversity of proposed strategies for the modification of the implant surfaces are presented and tested up to the *in vivo* stage. Numerous animal models have been utilized to evaluate the host response to endosseus implants, such as rabbits [31, 78, 81, 83-85, 87, 88, 90, 92, 94] , mini-pigs [80, 82, 91] , dogs [79, 86] , sheeps [44, 89] , goats [95] and pigs [77]. From these, the most commonly used animals are rabbits.

Prior to the clinical stage, animal studies are the most important step during the implant development since it will give important clues about the complex tissue reactions to the surface of material. However, it is quite difficult to establish a protocol that resembles the human implant site and the intra-oral environment under physiologic and pathologic conditions, as well as the availability of a large number of specimens over a long period of time required to evaluate proper osteointegration.

On the other hand, several different animal models, surgical sites, studied time-points and, control surfaces are presently being used making a straight comparison between the studies practically impossible.

4.2.2. *In vitro* studies

In a study by Yang *et al*.[97], a fast electrochemical anodization treatment applying different anodic currents was used in order to produce a nano/submicron-scale network oxide layer on Ti dental implant surface. This TiO2 network layer significantly enhanced the blood coagulation and human bone marrow stem cell adhesion on Ti surface.

Chiang *et al.* [96] hypothesized that a nano-scale oxide structure produced by electrochemical anodization on a titanium surface could improve cell growth. The results of the *in vitro* tests revealed higher cell growth on the anodized specimens than on the non treated surfaces. These results are quite predictable as it is widely accepted that rough implant surfaces show better performance than machined ones, so it would be more interesting to further compare this anodized nano-network surface with other surfaces commercially available.

Both previously cited authors emphasized their study in the nanofeatured surfaces obtained by anodization and successfully proven their bioactivity [96, 97].

Le Guehennec *et al*.[54] compared osteoblastic cell behavior on various titanium implant surfaces such as mirror-polished (Smooth-Ti), alumina-blasted and acid-etched (Alumina–Ti), SLA (sand-blasted, large-grit, acid-etched; supplied by Straumann AG) and biphasic calcium phosphate grit-blasted and acid-etched (BCP–Ti) titanium. The results showed that osteoblastic cells attach, spread and proliferate more rapidly on smooth surfaces than on rough surfaces while their differentiation was enhanced by rough morphologies. These *in vitro* results were related to higher implant survival rates in clinical practice for roughened implants as compared with machined surfaces. Although lower at the early culture time, cell viability increased more rapidly on SLA and BCP–Ti than on Alumina–Ti and Smooth-Ti. Biocompatible and resorbable BCP media was used as blasting material for obtaining a roughness for BCP–Ti comparable with that of Alumina–Ti but precluding the alumina contamination. However, their surface energies were different as the Alumina–Ti surface was more hydrophilic and favorable to the adhesion of osteoblastic cells , but lower in

proliferation than the BCP–Ti surface . Concerning the rough surfaces, all tested surfaces were cytocompatible regardless of the blasting material used. However, better cell behavior was showed on BCP-Ti and SLA surfaces than on Smooth and Alumina-Ti surfaces. Further *in vivo* studies are necessary for a better comparison of the osteointegrative properties of these surfaces and evaluate the exact effect of alumina contamination. In specific case of the BCP-Ti surface, it is mandatory to evaluate long-term performance, as the exact effects of a resorbable surface in the long-term performance of a dental implant is still unknown

Park et al. [93] focused their study on the biocompatibility and osteoinductive effect of a newly developed hydroxyapatite coating surface technique using aerosol deposition without pos-heating treatment and compared it with the same surface with post-heating treatment and a machined surface.

In order to coat HA layers on the surfaces of Ti substrates, various techniques have been used, including sputtering, electron beam deposition, plasma spraying, laser deposition, electrophoretic deposition, sol–gel coating, or biomimetic coating [41, 67]. With an exception of biomimetic coating, all of these methods require a post-heat treatment processing to obtain HA crystallization, because the uncrystallized HA coating will be easily dissolved and can prevent bone formation.

Aerosol deposition is a newly developed novel technique that has the potential to create a dense and uniform thin film without changing the original starting raw materials. This study revealed that HA coating by aerosol deposition is biocompatible, independent of treatment of post-heating, making this new coating aerosol deposition a simple and controllable method that results in the formation of a dense and nano-crystalline HA on implant surfaces with no composition change or phase transition.

Moreover, this study suggests a successful outcome using HA coating technique without post-heating treatment if aerosol deposition method is used. We considered only the *in vitro* results of this study, as the sample of the *in vivo* study was smaller than the inclusion criteria stipulated.

The HA coatings are a current topic of interest among the dental implant surface investigations in both *in vivo* [77, 78, 81, 84, 87, 88] and *in vitro* [93] stages due to their exelent bioactivity [64]. However, the long-term evaluation of these surfaces is mandatory in order to further commercialize them.

Elias *et al.* [101] evaluated the effect of fluoride treatments of cpTi samples on the adhesion and proliferation of osteoblastic cells on surfaces with and without cells fibronectin coating. Fluoride ion-modified implants have proven superior to sandblasted surfaces in terms of osteointegration, ultimately increasing the removal torques and fibronectin is a major extracellular matrix protein that is for its ability to promote cell adhesion, migration, proliferation, differentiation.

The results of this study show that the FN is critical to the biocompatibility of titanium surfaces. However, in the absence of this protein, the acid-etched and fluoride treated surface showed better results than the acid etched surface. The preparation of bioactive titanium surfaces via fluoride and/or fibronectin retention proved to be a useful surface modification approach to optimize and accelerate the osteointegration process for dental implants.

Dean *et al.* [98] determined whether exogenous arachidonic acid or prostaglandin E2 regulate osteoblast response to implant surface roughness. The study was based on the fact that systemic hormones have been shown to influence osteoblast response to implant surface roughness and that arachidonic acid metabolites, such as prostaglandin E2, are involved in the inflammatory phase of bone healing and subsequent bone remodeling. The results demonstrate that both AA and PGE 2 affect osteoblast response to Ti surface roughness. Arachidonic Acid did not only modify the surface roughness effect, but it also had the potential to reduce or eliminate it. The PGE 2 treatment also had an effect on cell response to surface roughness, although it was not as intense as that observed with AA.

Prostaglandins have an inhibitory effect on osteoclasts, but when given for extended periods of time, they stimulate bone resorption by increasing replication and differentiation of new osteoclasts. At relatively low

concentrations, the replication and differentiation of osteoblasts is stimulated and bone formation is increased. At high concentrations, PGE 2 inhibits collagen synthesis by osteoblasts and increases the bone-resorbing activity of osteoclasts. These multiple and biphasic effects of PGE 2 on bone has made it difficult to clearly elucidate the mechanism of action of this cytokine. Yet, further studies may clarify the actual potential of this hormone in the osteointegration process.

Miron *et al.* [99] showed that EMD (Emdogain -Enamel Matrix Protein) enhanced osteoblast differentiation on Ti surfaces, in a topography-independent manner. Emdogain is an enamel matrix protein derivative (EMD) extracted from developing porcine teeth, the major component of which are amelogenins, a family of hydrophobic proteins that account for more than 90% of the total protein content [105]. Although the idea of pre-coating EMD onto implant surfaces is still in its infancy, the results from this study emphasize that it could prove to be an important tool for enhancing bone formation around dental implants as it has the ability to control osteoblast proliferation and differentiation on titanium surfaces. For this matter, a large amount of *in vivo* and *in vitro* tests must be performed in order to explore the potential of this innovative application for Emdogain.

Yang *et al*. [100] designed a Simvastatin-loaded porous titanium implant surface and investigated the cell responses to the drug-loaded surface. Simvastatin is a commonly prescribed drug to reduce cholesterol concentrations and thereby, reduce heart attack risk [41] and recent studies have confirmed their potential in the increase of bone formation and suppression of osteoclast activity in the alveolar bone [106, 107]. It was demonstrated that a relatively low concentration of Simvastatin might be the optimal concentration for accelerating the osteogenic differentiation of pré-osteoblasts and therefore, have the potential to improve the nature of osteointegration. To date, few studies have reported the Simvastatin use related dental implants, and most were limited to systemic administration. In order to realize the potential of this drug-loaded surface and their impact in dental implantology, further investigations must be performed.

Sista *et al*. [102] Examined the biological behavior of mouse calvarial osteoblasts on pure titanium and two titanium based alloys namely, titanium-zirconium and titanium- nobirium by measuring the surface energy and roughness of these materials and compared the adhesion, proliferation and differentiation on them. The results indicate that the TiZr alloy, with the highest surface energy, is superior to pure Ti or TiNb alloy with respect to the biological properties of these materials, which further emphasize the role of surface energy of the substrates in regulating osteoblast cell behavior. This study indicates that TiZr has a better biological profile than Ti or TiNb based on the initial attachment of osteoblast cells on these materials. In order to fully elucidate the bioactive potential of TiZr, further *in vitro* and *in vivo* studies must be performed.

In vitro studies consist of a mandatory step in the development and evolution of a novel dental implant surface [30]. In laboratory models, several parameters of osteointegration are compared with control groups that often consist in machined surfaces. In order to assess the potential of a new biomaterial, this studies focus on tracking adhesion, morphology, proliferation, or cellular apoptosis. These parameters indicate as a preliminary state if whether the surface being tested has an adequate biocompatibility or not. However, results obtained in cell culture studies have not yet been fully correlated to *in vivo* studies due the difficulty in reproducing physiologic loading conditions and characteristics of the intra-oral environment. In addition, the bone organ culture studies of hard tissue integration is a quite problematic task as it requires the establishment and maintenance of cell cultures for long periods of time. Nevertheless, it is recognized that although the current limitations of *in vitro* studies, they represent an essential phase of study when developing a new dental implant surface.

Conclusions and future directions

5. Conclusions and future directions.

Over the past decade, several techniques to modify the implant surfaces have been widely studied and developed in an attempt to increase the rate of bone healing and to achieve rapid osteointegration. An appropriately modified titanium surface might certainly by the key factor for achieving a fast and stable implant outcome through optimal osteointegration.

The implant-to-bone interface should be able to promote the apposition of new bone by a proper triggering of the biochemical functions. In a similar way, the surface of the implant area in contact with the gingival tissues should enhance the apposition of soft tissues, sealing the way to the ingress, proliferation, and colonization of bacteria from the oral cavity. Besides implant design and surgical technique, surface chemical composition and topography are recognized to be key factors for achieving a fast and durable osteointegration, as well as for implant stability over time. The optimal surface roughness and texture is still a debated factor. However, there is a consensus that implants with rough surface have a better performance than smooth machined implants. Together with topography, the chemical properties of the implant surface in contact with the biologic tissues are recognized to play a fundamental role in the healing process, but the exact mechanism underlying the osteointegration process remains poorly understood.

Within the timeframe of the present review (since 2007), there have been number of dental implants commercially available with a wide variety of surface characteristics, both in terms of structural and chemical properties. Most of the *in vivo* and *in vitro* studies showed several novel dental implant surfaces, mostly consisting in modifications of the commercially available ones. One of the main drawbacks in the dental implant surface is the empirical nature of the manufacturing process as it lacks of consensus in the choice of uniform standard for obtaining controlled topographies or chemistries. For this matter, several *in vivo* and *in vitro* studies are required, but often performed without a hierarchical approach and standardized parameters using different surfaces, cell populations or animal models. There is an urgent need for more

fundamental research in this area that would normalize and combine both *in vitro* and *in vivo* studies ultimately leading to the appropriate clinical application.

A large amount of studies compare a specific rough surface with machined or turned surfaces as a control group. Since it is widely acknowledge that rough surfaces have better performance than machined or turned surfaces, the results have typically the tendency to be positive. Therefore, the inclusion of a widely accepted positive control would be beneficial to evaluate the performance of a certain surface in a more realistic way.

Clinical trials comparing different commercially available implant surfaces under similar clinical situations are rarely disclosed, making the outcome assessment between different surfaces quite difficult.

The use of nanotechnologies and the release of biologically active substances from dental implant surfaces, constitute promising routes as they may represent a benefit from both roughness and chemistry viewpoints and the possibility for a higher degree of control processes. However, in order to develop new generation of dental implants, key gaps in basic knowledge must be closed, and a series of prototype dental implants with increasing functionalities must be created. On the other hand, the information about the exact biological mechanisms that take place in the bone-to-implant interface is needed so that the relationships linking composition and materials architecture at scales of multiple lengths with macroscopic mechanical behavior could be established. The capability for osteogenesis and how an implant surface can be influenced and controlled by specific surface parameters must be undercover. In power of this valuable information, these relationships must be tested and evaluated systematically *in vitro* and *in vivo* and finally in clinical studies, maintaining the same rigorous analytic parameters and outcome measures. After the basic mechanism and sequence of events in the osteoitegration process are fully defined, this important knowledge could be used to design new implant systems capable of controlling the chemistry and cellular responses down to the molecular level. The ultimate goal would be the design of a custom-made implant according to patient´s own biology, needs and clinical indications.

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Appendix

5.1. List of tables

5.2. List of graphics